ATENT COOPERATION TR Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 July 1999 (06.07.99)	
International application No. PCT/US98/23705	**Applicant's or agent's file reference** REDC-710 PCT
International filing date (day/month/year) 06 November 1998 (06.11.98)	**Priority date** (day/month/year) 07 November 1997 (07.11.97)
Applicant KRANTZ, Alexander et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 May 1999 (21.05.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

PATENT COOPERATION TREATY

RECEIVED
JUN 25 1999
LIMBACH & LIMBACH

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:
WARD, Michael R.
LIMBACH & LIMBACH L.L.P.
2001 Ferry Building
SAN FRANCISCO, CALIFORNIA 94111-4262
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	22.06.99
Applicant's or agent's file reference: REDC-710 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 98/23705	06/11/1998	07/11/1997

Applicant: CONJUCHEM, INC. et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

21/05/1999

2. This date of receipt is:

- [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Danielle Wahrheit Telephone No. -2651

Form PCT/IPEA/402 (July 1998) P20452 (16/06/1999)

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

PCT/US98/23705

RECEIVED

MAY 2 1 1999

LIMBACH & LIMBACH

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

WARD, Michael, R. /PCT
Limbach & Limbach L.L.P.
2001 Ferry Building
San Francisco, CA 94111-4262
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 10 May 1999 (10.05.99)	
Applicant's or agent's file reference REDC-710 PCT	**IMPORTANT NOTIFICATION**
International application no. PCT/US98/23705	**International filing date** (day/month/year) 06 November 1998 (06.11.98)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
CONJUCHEM, INC. Suite 810 1801, boulevard de Maisonneuve West Montreal, Quebec H3H 1J9 Canada	CA	CA
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
CONJUCHEM, INC. Suite 3950, Third Floor 225 President Kennedy Avenue West Montreal, Quebec H2X 3Y8 Canada	CA	CA
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Aino Metcalfe
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

002610044

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

PCT

RECEIVED

JAN 1 3 1999

Limbach & Limbach

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

From the INTERNATIONAL BUREAU

To:

WARD, Michael, R. 1 PCT
Limbach & Limbach L.L.P.
2001 Ferry Building
San Francisco, CA 94111-4262
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
04 January 1999 (04.01.99)	
Applicant's or agent's file reference	**International application No.**
REDC-710 PCT	PCT/US98/23705

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

CONJUCHEM, INC. (for all designated States except US)
KRANTZ, Alexander et al (for US)

International filing date	:	06 November 1998 (06.11.98)
Priority date(s) claimed	:	07 November 1997 (07.11.97) 13 March 1998 (13.03.98)
Date of receipt of the record copy by the International Bureau	:	28 December 1998 (28.12.98)
List of designated Offices	:	

AP :GH,GM,KE,LS,MW,SD,SZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
National :AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CU,CZ,DE,DK,EE,ES,FI,GB,GE,GH,GM,HR,HU,ID,IL,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,US,UZ,VN,YU,ZW

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [X] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

Aino Metcalfe

Telephone No. (41-22) 338.83.38

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

PCT/U

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

RECEIVED

WARD, Michael, R. JAN 2 8 1999
~~Limbach & Limbach L.~~L.P.
2001 Ferry BuildingLIMBACH & LIMBACH
San Francisco, CA 94111-4262
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 14 January 1999 (14.01.99)	
Applicant's or agent's file reference REDC-710 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US98/23705	International filing date (day/month/year) 06 November 1998 (06.11.98)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 07 November 1997 (07.11.97)
Applicant CONJUCHEM, INC. et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
07 Nove 1997 (07.11.97)	60/064,705	US	05 Janu 1999 (05.01.99)
13 Marc 1998 (13.03.98)	60/077,927	US	05 Janu 1999 (05.01.99)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Carlos Naranjo

Telephone No. (41-22) 338.83.38

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

PCT/U

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

RECEIVED

WARD, Michael, R. JAN 2 8 1999
~~Limbach & Limbach L~~.L.P.
2001 Ferry Building LIMBACH & LIMBACH
San Francisco, CA 94111-4262
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 14 January 1999 (14.01.99)	
Applicant's or agent's file reference REDC-710 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US98/23705	International filing date (day/month/year) 06 November 1998 (06.11.98)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 07 November 1997 (07.11.97)
Applicant CONJUCHEM, INC. et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
07 Nove 1997 (07.11.97)	60/064,705	US	05 Janu 1999 (05.01.99)
13 Marc 1998 (13.03.98)	60/077,927	US	05 Janu 1999 (05.01.99)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Carlos Naranjo

Telephone No. (41-22) 338.83.38

WO 99/24075
PCT/US98/23705

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:
WARD, Michael, R.
Limbach & Limbach L.L.P.
2001 Ferry Building
San Francisco, CA 94111-4262
ÉTATS-UNIS D'AMÉRIQUE

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 20 May 1999 (20.05.99)	
Applicant's or agent's file reference REDC-710 PCT	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US98/23705	06 November 1998 (06.11.98)	07 November 1997 (07.11.97)

Applicant
CONJUCHEM, INC. et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,EP,IL,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,GM,HR,HU,ID,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   20 May 1999 (20.05.99) under No. WO 99/24075

5/7/00 orig.

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

PCT/US98/23705

RECEIVED

JUL 14 1999

LIMBACH & LIMBACH

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

WARD, Michael, R. / PCJ
Limbach & Limbach L.L.P.
2001 Ferry Building
San Francisco, CA 94111-4262
ÉTATS-UNIS D'AMÉRIQUE

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year)
06 July 1999 (06.07.99)

Applicant's or agent's file reference
REDC-710 PCT

IMPORTANT INFORMATION

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US98/23705	06 November 1998 (06.11.98)	07 November 1997 (07.11.97)

Applicant
CONJUCHEM, INC. et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   AP :GH,GM,KE,LS,MW,SD,SZ,UG,ZW
   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,BR,CA,CN,CZ,DE,GB,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
   National :AL,AM,AT,AZ,BA,BB,BY,CH,CU,DK,EE,ES,FI,GE,GH,GM,HR,HU,ID,IS,KE,
   KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MW,MX,PT,SD,SG,SI,SL,TJ,TM,TR,TT,UA,
   UG,UZ,VN,YU,ZW

   30mo date 5/7/2000 on cal

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, ~~if prescribed,~~ a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
F. Baechler

Telephone No. (41-22) 338.83.38

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

PCT

receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

Applicant's or agent's file reference *(if desired) (12 characters maximum)* REDC-710 PCT

Box No. I TITLE OF INVENTION

AFFINITY MARKERS FOR HUMAN SERUM ALBUMIN

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CONJUCHEM, INC.
225 President Kennedy Avenue West
Third Floor, suitre 3950
Montreal, Quebec H2X 3Y8
Canada

☐ This person is also inventor.

Telephone No.
(514) 844-5558

Facsimile No.
(514) 844-1119

Teleprinter No.

State *(that is, country)* of nationality:
CA

State *(that is, country)* of residence:
CA

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

KRANTZ, Alexander
847 North Humboldt Street, #310
San Mateo, California 94401
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:
US

State *(that is, country)* of residence:
US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

WARD, Michael R.
LIMBACH & LIMBACH L.L.P.
2001 Ferry Building
San Francisco, California 94111-4262
United States of America

Telephone No.
(415) 433-4150

Facsimile No.
(415) 433-8716

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HUANG, Wolin
872 Arcturus Circle
Foster City, California 94404
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HANEL, Arthur M.
195 Eureka Street, #5
San Francisco, California 94114
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

---

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HOLMES, Darren L.
3450 Drummond Street
Montreal, Quebec H3G 1T3
Canada

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BRIDON, Dominique P.
243 Chemin Cote Ste-Catherine
Outremont, Quebec H2V 2B2
Canada

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: FR

State *(that is, country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

---

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked):*

Regional Patent

☒ **AP ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line):*

☒ **AL** Albania .....
☒ **AM** Armenia .....
☒ **AT** Austria .....
☒ **AU** Australia .....
☒ **AZ** Azerbaijan
☒ **BA** Bosnia and Herzegovina .....
☒ **BB** Barbados
☒ **BG** Bulgaria .....
☒ **BR** Brazil .....
☒ **BY** Belarus
☒ **CA** Canada
☒ **CH and LI** Switzerland and Liechtenstein
☒ **CN** China .....
☒ **CU** Cuba .....
☒ **CZ** Czech Republic .....
☒ **DE** Germany .....
☒ **DK** Denmark .....
☒ **EE** Estonia .....
☒ **ES** Spain .....
☒ **FI** Finland .....
☒ **GB** United Kingdom
☒ **GE** Georgia .....
☒ **GH** Ghana .....
☒ **GM** Gambia
☒ **GW** Guinea-Bissau .....
☒ **HR** Croatia
☒ **HU** Hungary .....
☒ **ID** Indonesia
☒ **IL** Israel .....
☒ **IS** Iceland
☒ **JP** Japan .....
☒ **KE** Kenya .....
☒ **KG** Kyrgyzstan .....
☒ **KP** Democratic People's Republic of Korea .....
.....
☒ **KR** Republic of Korea .....
☒ **KZ** Kazakhstan .....
☒ **LC** Saint Lucia
☒ **LK** Sri Lanka
☒ **LR** Liberia
☒ **LS** Lesotho .....
☒ **LT** Lithuania
☒ **LU** Luxembourg
☒ **LV** Latvia
☒ **MD** Republic of Moldova .....
☒ **MG** Madagascar .....
☒ **MK** The former Yugoslav Republic of Macedonia .....
.....
☒ **MN** Mongolia
☒ **MW** Malawi .....
☒ **MX** Mexico .....
☒ **NO** Norway
☒ **NZ** New Zealand .....
☒ **PL** Poland .....
☒ **PT** Portugal .....
☒ **RO** Romania
☒ **RU** Russian Federation .....
☒ **SD** Sudan
☒ **SE** Sweden
☒ **SG** Singapore
☒ **SI** Slovenia .....
☒ **SK** Slovakia .....
☒ **SL** Sierra Leone
☒ **TJ** Tajikistan .....
☒ **TM** Turkmenistan .....
☒ **TR** Turkey .....
☒ **TT** Trinidad and Tobago .....
☒ **UA** Ukraine .....
☒ **UG** Uganda .....
☒ **US** United States of America .....
.....
☒ **UZ** Uzbekistan .....
☒ **VN** Viet Nam .....
☒ **YU** Yugoslavia .....
☒ **ZW** Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☐ .....
☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:** in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V., the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed:** in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, the **earlier application is an ARIPO application:** in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non-prejudical disclosures or exceptions to lack of novelty" and furnish that statement below.*

LIMBACH, Karl A.
LIMBACH, George C.
UILKEMA, John K.
SMITH, Neil A.
DEVITT, Veronica C.
YIN, Ronald L.
SEKIMURA, Gerald T.
STALLMAN, Michael A.
GIRARD, Philip A.
POLLOCK, Michael J.
EVERETT, Stephen M.
EQUITZ, Alfred A.
DALLA VALLE, Mark A.
PICKERING, Mark C.
FROST, Kathleen A.
HODES, Alan S.
SAMMUT, Charles P.
COLEMAN JAMES, Patricia
LIMBACH, Alan A.
LIMBACH, Douglas C.
KEATING, Brian J.
OH, Seong-Kun
KING, Cameron A.
MAEDA, Mayumi
TOBIN, Kent J.
WARD, Michael R.
SANTISI, Steven M.
HAMILTON, Charles L.
HOGLUND, Heath W.
McCARTHY, J. Thomas
SMITH, Andrew V.
GOLDMAN, William G.
HARRIEL, Kyla L.

All attorneys are members or associates of the firm LIMBACH & LIMBACH L.L.P. Address, telephone number, and facsimile number of all are indicated in Box IV.

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 7 November 1997	60/064,705	US		
item (2) 13 March 1998	60/077,927	US		
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1) and (2)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country (or regional Office)

Box No. VIII CHECK LIST: LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	5
description (excluding sequence listing part)	:	52
claims	:	7
abstract	:	1
drawings	:	18
sequence listing part of description	:	0
Total number of sheets	:	83

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☒ separate ~~signed~~ power of attorney (unsigned)
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as items(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Transmittal Letter; Acknowledgement Card

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

LIMBACH & LIMBACH L.L.P.

By Michael R. Ward
Michael R. Ward
Attorneys for Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

EXPRESS MAIL LABEL NO. EL387347871US
Attorney Docket No. REDC-710 USA

RECEIVED

JUN 21 1999

LIMBACH & LIMBACH

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL SEARCHING AUTHORITY

To:
LIMBACH & LIMBACH L.L.P.
Attn. WARD, M
2001 Ferry Building
SAN FRANCISCO, CALIFORNIA 94111-4262
UNITED STATES OF AMERICA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

US-105 due.
11/15/99

(PCT Rule 44.1)

Applicant's or agent's file reference REDC-710 PCT	Date of mailing (day/month/year) 15/06/1999
International application No. PCT/US 98/23705	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant CONJUCHEM, INC. et al.	International filing date (day/month/year) 06/11/1998

Due 8/15/99

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Claudia Aragone

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
REDC-710 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/ 23705	06/11/1998	07/11/1997
Applicant		
CONJUCHEM, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant m within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the fig

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/23705

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-54,56,57
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-55 in part

   Conjugates of a peptide, selective for albumin, and a therapeutic agent.

2. Claims: 1-55 in part

   Conjugates of a peptide, selective for albumin, and a diagnostic agent.

3. Claims: 56-57

   Method for screening for the affinity of a compound for human serum albumin.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1-54,56,57

In view of the large number of compounds, which are defined by the general definition in the claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see guidelines, Chapter III, paragraph 2.3).

INTE[illegible] .TIONAL SEARCH REPORT

[illegible]ational Application No
PCT/US 98/23705

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48 G01N33/68 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 121, no. 7, 15 August 1994 Columbus, Ohio, US; abstract no. 77796, INAGAWA, JUNICHI: "Biotin-derivatizing agent for labeling protein or DNA probes" XP002101950 see abstract -& JP 06 092968 A (NAKARAI TESUKU KK, JAPAN) --- -/--	1-55

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 May 1999	15.06.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dullaart, A

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SJOBRING U: "Isolation and molecular characterization of a novel albumin-binding protein from group G streptococci." INFECT IMMUN, SEP 1992, VOL. 60, NO. 9, PAGE(S) 3601-3608, XP002101944 see abstract see page 3601, right-hand column - page 3602, left-hand column see page 3603, left-hand column see figures 1-3,7 ---	1-55
Y	GHINEA N ET AL: "Endothelial albumin binding proteins are membrane-associated components exposed on the cell surface." J BIOL CHEM, MAR 25 1989, VOL. 264, NO. 9, PAGE(S) 4755-4758, XP002101945 see paragraph RESULTS AND DISCUSSION ---	1-57
Y	AHLFORS C.E. ET AL: "Plasma fluorescein binding and transcapillary fluorescein escape rate in renal failure associated with diabetes" AMERICAN JOURNAL OF KIDNEY DISEASES, 1995, VOL. 25, NO. 4, PAGE(S) 543-547, XP002101946 see page 543 ---	1-57
Y	NAGATAKI S. ET AL: "Binding of fluorescein monoglucuronide to human serum albumin" INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, 1985, VOL. 26, NO. 8, PAGE(S) 1175-1178, XP002101947 see abstract see paragraph RESULTS ---	1-57
Y	M. ROWLAND & T.N. TOZER: "Clinical Pharmacokinetics: Concepts and Applications" 1980 , LEA & FEBIGER , PHILADELPHIA, US XP002101949 see page 246 - page 265 ---	1-55
X	SCHNITZER J E ET AL: "ANTIBODIES TO SPARC INHIBIT ALBUMIN BINDING TO SPARC, GP60, AND MICROVASCULAR ENDOTHELIUM" AMERICAN JOURNAL OF PHYSIOLOGY: HEART AND CIRCULATORY PHYSIOLOGY, vol. 32, no. 6, 1 December 1992, pages H1872-H1879, XP000613858 see page H1873, left-hand column see page H1875, right-hand column - page H1877, left-hand column --- -/--	56,57

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RICHTER E ET AL: "FLUORO IMMUNO CYTO ADHERENCE A NEW METHOD FOR THE IDENTIFICATION AND ENUMERATION OF ANTIGEN BINDING CELLS" ZEITSCHRIFT FUER IMMUNITAETSFORSCHUNG, XP002101948 see page 354 - page 355 see table 1 ---	56,57
X	SJOLANDER A ET AL: "The serum albumin-binding region of streptococcal protein G: a bacterial fusion partner with carrier-related properties" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 201, no. 1, 14 February 1997, page 115-123 XP004050046 see paragraph 2.1 -----	56,57

M.H

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REC'D 01 FEB 2000

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
REDC-710 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/23705	06/11/1998	07/11/1997
International Patent Classification (IPC) or national classification and IPC A61K47/48		
Applicant CONJUCHEM, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/05/1999	28.01.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Giacobbe, S Telephone No. +49 89 2399 8463

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US98/23705

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-52 as published

Claims, No.:

1-57 as published

Drawings, sheets:

1/18-18/18 as published

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 2-57
	No:	Claims 1
Inventive step (IS)	Yes:	Claims 2-57
	No:	Claims 1
Industrial applicability (IA)	Yes:	Claims 1-57
	No:	Claims

2. Citations and explanations

see separate sheet

1. Section V

1.1 Art 33 (2), (3) and (4) PCT

The subject-matter of present claims 1-57 fulfills the requirements of Arts 33(2), (3) and (4) PCT.

None of the cited documents discloses or fairly suggests a compound having the structure and the properties of those claimed in independent claim 1. The application therefore inventively solves the technical problem of '*how to selectively transfer a therapeutic or diagnostic agent to human serum albumin*'. The same applies to the screening method described in independent claim 56.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:
A61K 47/48	A2	WO 99/24075
		(43) International Publication Date: 20 May 1999 (20.05.99)

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(30) Priority Data:

60/064,705	7 November 1997 (07.11.97)	US
60/077,927	13 March 1998 (13.03.98)	US

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(72) Inventors; and

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(74) Agents: WARD, Michael, R. et al.; Limbach & Limbach L.L.P., 2001 Ferry Building, San Francisco, CA 94111–4262 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: AFFINITY MARKERS FOR HUMAN SERUM ALBUMIN

(57) Abstract

Methods and compositions are provided for identifying compounds having affinity or complementarity to a target molecule. Compounds according to the invention may be described by the formula $E–C_a–R–C_b–A$, wherein E is a therapeutic or diagnostic agent, R is a reactive group, C_a and C_b are connector groups between E and R and between R and A, respectively, and A is a group having an affinity for human serum albumin, wherein affinity group A comprises a sequence of amino acid residues $–O_1–O_2–X_1–X_2–B$ in which the amino acid residues are independently selected from the group of all twenty naturally occurring amino acids. Compounds according to the invention may be used for labeling the target molecule, particularly where the target molecule is naturally found in a complex mixture, such as a physiological fluid, like blood. By affinity labeling *in vivo*, the lifetime of physiologically active entities can be greatly enhanced by becoming bound to long–lived blood components. The covalently bound entity may also serve as an antagonist or agonist of a particular binding protein or as an enzyme inhibitor.

FOR THE PURPOSES OF INFORMATION ONLY

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AFFINITY MARKERS FOR HUMAN SERUM ALBUMIN

RELATED APPLICATIONS

This application is a non-provisional application, claiming benefit under 35 U.S.C. § 119(e) of United States Provisional Application No. 60/064705, entitled BIOCONJUGATION AND AFFINITY GROUPS filed November 7, 1997 and United States Provisional Application No. 60/077927 entitled FIYEE AFFINITY MARKER filed March 13, 1998.

FIELD OF THE INVENTION

This invention relates to libraries of affinity markers, and especially FIYEE, for human serum albumin. This invention is further related to methods of screening the affinity marker libraries, identification of specific markers and the use of the markers as delivery agents for therapeutic *in vitro* and *in vivo* diagnostic agents.

BACKGROUND OF THE INVENTION

The advent of combinatorial chemistry has provided a platform for a wide variety of opportunities. The ability to produce large libraries of different compounds means that one can screen a large array of conformations, and charge distributions for their ability to bind to other compounds to act as agonists or antagonists, in binding to specific sites of a target protein, to investigate the conformation of a particular protein site, such as an enzymatic cleft or membrane channel protein, and the like.

The existence of combinatorial chemistry affords new opportunities towards new therapies for diseases for which prior drugs are inadequate or for which there are no present successful therapies. The presently available drugs are inadequate for a wide variety of reasons, in many cases, the pharmacokinetics of the drugs. Parenteral delivery in a bolus has many constraints on its efficacy. Since most drugs have serious side effects, one is constrained as to the upper dosage level. On the other hand, drugs may undergo enzymatic modification, oxidation, degradation in the liver, secretion, and the like, so that the lifetime of the drug will be limited by the various mechanisms which serve to diminish the effective level of the drug.

Drugs which have been approved for use with humans have already been shown to be generally safe at the prescribed dosage and effective. Therefore, there are substantial advantages in being able to modify these drugs, where their safety and efficacy are not unduly altered. In this way, the economies of dealing with known entities can be achieved, while at the same time increasing the available therapeutic opportunities.

BRIEF SUMMARY OF THE INVENTION

The present invention addresses these and other problems of the prior art by providing compounds having the formula E-C_a-R-C_b-A. In this formula, E contains an active diagnostic moiety or pharmacophore and suitable connectors, R is a potentially reactive functional group contained within the entity and is capable of transferring an active diagnostic moiety or pharmacophore. C_a and C_b are connector groups between E and R and between R and A, respectively. A is any molecule or part of a molecule that possess specific binding determinants for a target molecule, such as proteins including human serum albumin. (These components of compounds according to the present invention are defined more fully below.) In some compounds according to the invention, affinity group A comprises a sequence of amino acid residues -O_1-O_2-X_1-X_2-B in which the amino acid residues are independently selected from the group of all twenty naturally occurring amino acids. A particularly preferred sequence is F*I*YEE. Preferred compounds according to the invention include biotin-S-Ph-C(O)-F*I*YEE- NH_2, biotin-OPh-C(O)-F*I*YEE-NH_2, LC-biotin-S-Ph-C(O)-F*I*YEE-NH_2 , biotin-Gly-OPh-C(O)-F*I*YEE-NH_2, fluorescein-Gly-OPh-F*I*YEE-NH_2, LC-biotin-OPh-C(O)-F*I*YEE-NH_2, argatroban-AEA_3-βAla-Gly-OPh-C(O)-F*I*YEE-NH_2 and fluorescein-thiourea-AEA_3-Gly-OPh-C(O)-F*I*YEE-NH_2 , where LC is **?????** These compounds can bind to specific proteins in vivo and enhance the half lives of diagnostic and therapeutic agents as well as control side effects.

.....The present invention is also directed to methods for screening for the affinity of a compound for active sites on a target molecule. In methods according to the invention, the target molecule is immobilized on a test substrate and then incubated with the compound under conditions that support interaction (i.e., noncovalent

binding) between the compound and the active sites on the target molecule. Then, the interaction between the active sites and the compound is quenched. Thereafter, an assay can be performed to detect the activity of the target molecule.

.....This invention could oe seen as a replacement for some antibody uses, particularly in the field of in vitro diagnostic or in vivo therapy. The present invention is an improvement on US Patent 5 612 034 (Pouletty, et al.) teaching the art of labeling protein in vivo in a nonspecific manner since the present invention extends the life of the drug and controls its distribution, thereby limiting its side effects.

BRIEF DESCRIPTION OF THE DRAWINGS

This invention will be better understood by reference to the figures, in which:

Figure 1 shows the structural formula of the most preferred affinity group of the invention, -F*I*YEE.

Figure 2 shows a reaction scheme for the use of a preferred embodiment of the invention for covalently attaching biotin to HSA.

Figure 3 shows a schematic illustration of the construction of an affinity labeling library of the present invention.

Figure 4 shows optical density measurement for the screening of a library according to the present invention at the level of 81 compounds per well with a 1 minute quenching time

Figure 5 shows optical density measurement for the screening of a library according to the present invention at the level of 81 compounds per well with a 15 minutes quenching.

Figures 6a and 6b show optical density measurements for the screening of a library according to the present invention at the level of 9 compounds per well.

Figure 7 shows an immunoblot in which human serum and plasma samples were labeled with two different concentrations of biotin-OPh-CO- NH_2 to assess compound lack of specificity in the absence of F*I*YEE peptide.

Figure 8 shows a plot of optical density as a function of HO-Ph-CO- F*I*YEE-NH_2 concentration in an experiment to investigate competitive binding to HSA.

Figure 9 shows a plot of optical density as a function of HO-Ph-CO- F*l*YEE - NH_2 concentration in an experiment to investigate competitive binding to HSA as compared to biotin-OPh-CO-F*l*YEE- NH_2.

Figure 10 shows a plot of optical density as a function of time for a kinetic study by ELISA capture in which human plasma was treated with biotin-OPh-CO-F*l*YEE- NH_2, biotin-OPh-CO- NH_2, NHS-LC-biotin or biotin-BMCC (BMCC is 1-biotinamido-4-[4'-(maleimidomethyl)cyclohexanecarboxamido]butane).

Figure 11 shows a graph showing the rate of disappearance of biotin-OPh-CO-NH_2 and appearance of HO-Ph-CO- NH_2 as a result of reaction of HSA with biotin-OPh-CO- NH_2.

Figure 12 shows a plot of the concentration of HO-Ph-CO-F*l*YEE -NH_2 resulting from the reaction of biotin-OPh-F*l*YEE–NH_2 in commercial human plasma as a function of time.

Figure 13 shows a plot of the concentration of HO-Ph-CO- F*l*YEE -NH_2 and biotin-OPh-CO-F*l*YEE -NH_2 as a function of time during hydrolysis of biotin-OPh-CO-F*l*YEE- NH_2 in PBS (pH 7.4, RT).

Figure 14 shows a plot of the concentration of HO-Ph-CO- F*l*YEE -NH_2 as a function of time during reaction of biotin-OPh-CO- F*l*YEE- NH_2 with HSA in PBS (pH 7.4, RT).

Figure 15 shows HPLC chromatographs of tryptic digest of HSA (top) and HSA:LC-biotin (bottom).

Figure 16 is a reaction scheme setting forth the procedure for the synthesis of biotin-OPh-CO-F*l*YEE-NH_2 according to the invention.

Figure 17 is a reaction scheme setting forth the procedure for the synthesis of biotin-Gly-OPh-CO-F*l*YEE-NH_2 according to the invention.

Figure 18 is a reaction scheme setting forth the procedure for the synthesis of LC-biotin-OPh-CO-F*l*YEE-NH_2 according to the invention.

Figure 19 is a reaction scheme setting forth the procedure for the synthesis of argatroban-AEA_3-βAla-Gly-OPh-CO-F*l*YEE-NH_2 •2 TFA according to the invention.

Figure 20 is a reaction scheme setting forth the procedure for the synthesis of Fluorescein-thiourea-AEA-Gly-OPh-CO-F*l*YEE-NH_2 according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

In order to ensure a complete understanding of the invention, we first provide definitions of terms used in this patent. We then discuss the results of specific screening experiments used to determine affinity groups for labeling human serum albumin (HSA). We discuss the specific affinity groups identified by the screening and the specific entity, reactive groups, and connecting groups used in the library screening experiments. We then discuss more general target molecules, affinity groups, reactive groups, connecting groups and entities that fall within the scope of the invention. Finally, we present experimental results for the synthesis of the libraries used in the present invention together with the experimental screening results and the results of experiments characterizing the entity/target-molecule binding.

Definitions

Entity: a segment of an affinity labeling reagent that is to be covalently attached to the target molecule. Generally, the entity includes an active diagnostic moiety or pharmacophore and suitable connectors. Examples of possible diagnostic or therapeutic agents are provided below.

Affinity Group: any molecule or part of a molecule that posses specific binding determinants for a target molecule. In the context of this filing, the affinity group includes oligomeric moieties such as peptides, carbohydrates and nucleotides and combinations thereof that confer affinity to the target molecule by virtue of their complementarity for specific sites of the target. Such oligomeric units distinguish affinity groups from connectors and reactive groups, all of which are components of the entity in rate. Since the activities of affinity reagents are measured in terms of the rate by which they covalently label their targets, any increase in rate above 100% (factor of 2) conferred by such oligomeric units, relative to a standard molecule lacking such oligomeric units, relative to a standard molecule lacking an oligomeric group complementary to the target, defines these groups as affinity groups in this

application. According to the invention, suitable affinity groups for labeling human serum albumin include oligomeric molecules including peptides, carbohydrates and nucleotides and combinations thereof.

Reactive Group: generally a potentially reactive organic functional group contained within the entity capable of transferring an active diagnostic moiety or pharmacophore. Reactive groups according to the invention may be bonded to the affinity group by a first connecting group. The reactive group may be bonded to the entity by a second connecting group and in this case, the bond between the reactive group and the second connecting group must be able to cleave allowing the second connecting group to bond to the target molecule.

Reactive Functional Group: generally any group that may be incorporated in the molecules of the invention either between the reactive group and the affinity group or between the reactive group and the entity.

Target Molecules: any molecules to which affinity groups are complementary by virtue of specific binding determinants and to which the whole entity may react to form a covalent bond. Human serum albumin is the most preferred target molecule; examples of other preferred target molecules are discussed below.

In Vitro Diagnostic Agent: any molecule that finds use in *in vitro* diagnostic analysis.

In Vivo Diagnostic Agent: any molecule that finds use in *in vivo* diagnostic analysis.

Diagnostic Agents: Diagnostic agents include agents used in diagnostic techniques such as positron emission tomography (PET), computerized tomography (CT), single photon emission computerized tomography (SPECT), magnetic resonance imaging (MRI), nuclear magnetic imaging (NMI), fluoroscopy and ultrasound, fluorphors, chromophors and chemiluminophors.

Diagnostic agents of interest include contrast agents, radioisotopes of such elements as iodine (I), including ^{123}I, ^{125}I, ^{131}I, etc., barium (Ba), gadolinium (Gd), technetium (Tc), including ^{99}Tc, phosphorus (P), including ^{31}P, iron (Fe), manganese (Mn), thallium (Tl), chromium (Cr), including ^{51}Cr, carbon (C), including ^{11}C, or the like, fluorescently labeled compounds, and fluorine containing chemicals useful in ultrasound, etc.

Therapeutic Agent: any molecule that has a therapeutic (i.e., healing or curative) effect. Therapeutic agents are agents that have a therapeutic effect. Therapeutic agents include drugs, antibiotics, antiinfectives, anti-cancer agents, pain management agents, cardiovascular drugs (antithrombotics, anticoagulants, anti-platelet, protease inhibitors, calcium channel blockers, etc.), anti-inflammatory agents, antiproliferative drugs, oligonucleotides (sense and antisense). Therapeutic agents are generally drugs or small molecules having a molecular weight less than 2000.

Specific Affinity Groups for Human Serum Albumin

Using library screening techniques according to the invention and exemplified below, the inventors have identified a series of compounds that are useful for attaching an entity (usually a diagnostic or therapeutic agent) to human serum albumin (HSA). In one embodiment of the present invention, the compounds have the formula, E-C_a-R-C_b-A, where E is the entity to be attached to the target molecule, C_a and C_b are connecting groups, R is a reactive group and A is an affinity group. C_b and C_a are also referred to herein as the first connecting group and the second connecting group, respectively. As discussed below, the connecting groups C_a and C_b may not be required and the invention also covers compounds of the formula E-R-C_b-A, E-C_a-R-A and E-R-A.

As exemplified below, the inventors synthesized an affinity labeling library of general formula E-R-O_1-O_2-X_1-X_2-B-NH_2 and screened this library of compounds against HSA as the target molecule. For the diagnostic screening, E was a biotin group; R was selected from –S-Ph-C(O)- (example 1 below), -O-Ph-C(O)- (example

2) and –N-Ph-C(O)- (example 3); and O_1, O_2, X_1, X_2 and B are amino acid residues selected from glutamic acid, glutamine, arginine, methionine, serine, tyrosine, leucine, phenylalanine and tryptophan. The inventors also synthesized an embodiment of the invention of the formula E-C_a-R-O_1-O_2-X_1-X_2-B-NH_2 where E is biotin and C_a is –NH-$(CH_2)_5$-C(O)-.

The library screening results indicated that library members exhibiting enhanced binding to HSA included the following amino acid residues:

O_1 is selected from phenylalanine, arginine, glutamine, tyrosine, glutamic acid and tryptophan;

O_2 is selected from leucine, arginine, glutamic acid, tryptophan and phenylalanine;

X_1 is selected from phenylalanine, tryptophan, methionine and tyrosine;

X_2 is selected from serine, arginine and glutamic acid; and

B is selected from serine, arginine and glutamic acid.

Analysis of these results suggest that hydrophobic residues are preferred at both the O_1 and O_2 positions, with large hydrophobic residues being more preferred; hydrophobic residues are also preferred at the X_1 position. Although the results do not appear to demonstrate any clear preference for different residues at the X_2 and B positions, based on other considerations discussed below, it appears that more polar, charged amino acids are preferable.

To reduce the possibility of degradation of a peptide affinity group (i.e., where two or more of the residues in O_1-O_2-X_1-X_2-B are amino acids), one or more D-amino acid residues can be included in an L-amino acid peptide or one or more L-amino acid residues can be included in an D-amino acid peptide. For example, including a D-amino acid residue at position O_2, X_1, or X_2 in an L-amino acid peptide (or vice versa) will yield two D-L bonds, which may reduce degradation. In a preferred embodiment of this invention , the inventors included a D-amino acid residue at the O_2 position and included L-amino acid residues at the other positions.

Preferred combinations of the O_1 and O_2 residues are as follows:

O_1 is phenylalanine and O_2 is D-leucine;

O_1 is arginine and O_2 is D-arginine;

O_1 is glutamine and O_2 is D-glutamic acid;

O_1 is glutamic acid and O_2 is D-tryptophan;

O_1 is tryptophan and O_2 is D-tryptophan; and

O_1 is tryptophan and O_2 is D-glutamic acid.

In the most preferred combination, O_1 is phenylalanine and O_2 is leucine as determined by the analysis of Figure 4. Figure 4 represents the rate of addition, expressed as optical density units, of library members on HSA. An elevated value for the optical density is representative of a larger amount of biotin on HSA at a given time. A larger amount of biotin on HSA at a given time is representative of a higher specificity of a library member or members for a given site, resulting in an accelerated addition of biotin on the target site.

The preferred amino acids at the X_1, X_2, and B positions are tyrosine, glutamic acid and glutamic acid respectively. The most preferred affinity group is -F*l*YEE, where F is phenylalanine, *l* is D-leucine, Y is tyrosine, and E is glutamic acid.

In a preferred embodiment, the B position amino acid residue is the C-terminal amino acid. In another preferred embodiment, the B position amino acid residue exists in its carboxamide form rather than in the carboxylic acid form. In this preferred embodiment the –F*l*YEE affinity group has the structural formula shown in Figure 1.

The screening experiments described herein identified the affinity groups discussed above and these screening results indicate that the affinity groups may preferentially bind to HSA and may therefore be useful in directing specific entities to covalently bind to HSA. Specific experiments demonstrating such specific binding are described in the EXPERIMENTS section.

Reactive Groups (R)

A preferred affinity marker compound of the present invention includes a reactive group (R) in addition to the affinity group described above. The reactive group may be connected through to the affinity group via a first connecting group (C_b) or, preferably, is bonded directly to the affinity group.

Generally the reactive group, R, will include a reactive functional group selected from carboxy, phosphoryl, alkyl esters, thioesters, phosphoesters, ortho esters, imidates, mixed anhydrides, disulphides, amides and thioamines. It is preferred that the reactive group is stable in an aqueous environment. In a preferred embodiment, the reactive group will include an aromatic moiety, preferably a substituted or unsubstituted phenyl group.

Generally, the bond between the reactive group and the affinity group should be a thermodynamically stable, but potentially reactive covalent bond (i.e., irreversible bond). In a preferred embodiment of the invention, the reactive group is bonded directly to the O_1 amino acid of the affinity group. In a more preferred embodiment, the reactive group is bonded to the O_1 amino acid of the affinity group by an amide linkage.

In one embodiment of the invention, the reactive group has the formula $-X-R_1-C(O)-$, where X is sulfur, oxygen, or nitrogen; R_1 includes a substituted or unsubstituted aromatic group; and C(O) represents a carboxyl group. Also suitable is a reactive group of the formula $-X-R_1-C(S)-$, where X is sulfur, oxygen, or nitrogen; R_1 includes a substituted or unsubstituted aromatic group; and C(S) is a thiocarbonyl group. Preferably, X is sulfur or oxygen. In another preferred embodiment, X is directly bonded to an aromatic carbon in the R_1 group. In a more preferred embodiment, R_1 is a substituted or, preferably, an unsubstituted phenyl. In this context, by "substituted phenyl", we mean a phenyl group bearing substituents in addition to the –X- and –C(O)- groups explicitly laid out in the formula. In a more preferred embodiment, R_1 is unsubstituted phenyl and the –X- and –C(O)- substituents are bonded to the phenyl group in a para configuration. In another preferred embodiment of the invention, the reactive group carboxyl group is directly bonded to the O_1 amino acid of the affinity group, generating a stable amide bond.

In another embodiment of the invention, the reactive group is attached to the affinity group via a first connecting group. In this embodiment, suitable connecting groups may be as described below in the "General Connecting Groups" section.

Entity (E)

The reactive group may either be bonded directly to the entity or connected thereto via a second connecting group (C_a). The entity may generally be any fragment or moiety that is desired to be covalently bonded to the target molecule and preferred entities include a variety of pharmacophore and active diagnostic agents that are described in detail in the "General Entities" section below. In a preferred embodiment, the entity includes a biotin group.

When compounds according to the invention covalently react with to the target molecule, the covalent bond between the entity and the reactive group cleaves, liberating an -RL-A or -RL-C_b-A fragment, wherein RL is R after reaction with a nucleophile, whereby an entity/target-molecule covalent bond forms to attach the entity to the target molecule. This mechanistic understanding of the chemistry is useful in determining which entity/reactive-group bonds are most suitable. In a preferred embodiment of the invention, the entity is bonded to the reactive group by an amide, or more preferably, an ester or thioester bond.

In a preferred embodiment of the invention, the entity forms a covalent bond to an amino group on the target molecule and in this case an ester or thioester bond between the entity and the reactive group is preferred. However, in other embodiments of the invention, the entity can form covalent bonds to hydroxyl, thiol, or other available functional groups on the target molecule.

If the entity is to form a covalent bond with a hydroxyl group on the target molecule, it is preferred that the entity bonds to the reactive group by an ester bond. If the entity is to form a covalent bond with a thiol group on the target molecule, it is preferred that the entity is bonded to the reactive group by a thioester or disulfide bond.

Second Connecting Group (C_a)

Typical connecting groups that may be used are described in detail in the "General Connecting Groups" section below. In a preferred embodiment, the second connecting group LC (for "long chain") has the formula –NH-$(CH_2)_n$-C(O)-, where n is an integer between 1 and 25. In a more preferred embodiment, the second

connecting group has the formula –NH-$(CH_2)_5$-C(O)- and –NH-CH_2-C(O)- . When a second connecting group is present, the mechanism for bonding the entity to the target molecule includes the breaking of the bond between the second connecting group and the reactive group and the formation of a bond between the second connecting group and a functional group on the target molecule. In this case, the entity will be attached to the target molecule via the second connecting group. One effect of the presence of the second connecting group is that the entity may be held sterically clear of the target molecule after it has been covalently attached; that is, the entity ends up tethered to the target molecule via the second connecting group. This aspect of the invention may be important in maintaining the activity of a therapeutic agent that may lose activity if it is too closely attached to the target molecule.

Preferred Embodiments

The following compounds are preferred embodiments of the present invention: A compound selected from the group consisting of biotin-S-Ph-C(O)-FlYEE- NH_2, biotin-OPh-C(O)-FlYEE-NH_2, LC-biotin-S-Ph-C(O)-FlYEE-NH_2 , biotin-Gly-OPh-C(O)-FlYEE-NH_2, fluorescein-Gly-OPh-FlYEE-NH_2, LC-biotin-OPh-C(O)-FlYEE-NH_2, argatroban-AEA_3-βAla-Gly-OPh-C(O)-FlYEE-NH_2, and fluorescein-thiourea-AEA_3-Gly-OPh-C(O)-FlYEE-NH_2.

Use of Preferred Embodiments to Attach the Entity to HSA

Figure 2 shows the reaction scheme for the use of a preferred embodiment of the present invention **5** to attach a biotin group **10** (the entity) to HSA **40** (the target molecule). In the preferred embodiment shown in Figure 2, the reactive group **20** is an -O-Ph-C(O)- group and the affinity group **30** is -F*l*YEE. The entire molecule composed of 5, 10, 60, 20 and 30 is referred to here as an affinity labeling reagent or drug affinity label conjugate. To form the target-molecule/entity complex **50**, the entity/reactive functional group bond **60** cleaves and an amine group **70** on the target molecule **40** forms an amide bond **80** via a $B_{AC}2$ mechanism with the entity **10**. The reactive-group/affinity-group molecule **90** is liberated. The specific experimental

conditions and product characterization results for this reaction are described in detail in the "Examples " section.

General Target Molecules

Blood proteins are an important class of target molecules. Target proteins may be long lived in that they have a half life of at least about 12 hours, preferably at least about 48 hours, more preferably at least about 5 days. Preferred target proteins, either individually or as part of cells, include the surface membrane proteins of erythrocytes, particularly glycophorin A and B and 3, and C, T or B cell surface proteins, such as CD3, B7, p28, CTLA-4, CD34, Thy1, CD4, CD8, LFA1, CD5, sIgE, sIgM, platelet proteins, such as IIb/IIIA, leukocyte surface membrane proteins, serum albumin, immunoglobulins, particularly IgG and IgM, apolipoproteins, such as LDL, HDL and VLDL, and proteins associated with chylomicrons, endothelial cell surface proteins, such as integrins, adhesion proteins, etc. Cells for targeting according to the invention include platelets, erythrocytes, endothelial cells, T cells or subsets thereof, B cells or subsets thereof, other leukocytes, such as macrophages, monocytes, neutrophils, basophils, NK cells, eosinophils, stem cells, such as hematopoietic stem cells, tumorous or malignant cells, infected cells, such as virally infected, e.g., retroviruses such as HIV, DNA viruses, such as hepatitis B or C virus, etc. The cells may be fixed or mobile.

A number of proteins of interest have numerous reactive functionalities for reaction *ex vivo* or *in vivo*. For example, human serum albumin, glycophorins, thrombin, adenylate kinase, plasminogen, β-lactamase, ACE, glutathione transferase, HMG CoA reductase, gastric lipase, and lecithin:cholesterol acyl transferase have active amino groups for conjugation. Cathepsin cysteine proteases and cytosolic phospholipase A_2 have numerous thiol groups for conjugation.

General Affinity Groups

For the most part, the affinity group A will be oligomeric and, therefore, various types of affinity groups which find use in the invention include, for example, oligopeptides, oligonucleotides, oligosaccharides, combinations thereof, or the like.

Affinity groups exhibit an affinity through non-covalent binding and association with biologically active molecules. Generally, the affinity groups represented in any particular library will be of a common type. Oligomeric affinity groups will be characterized by their ready synthesis as a combinatorial library, so that the synthetic chemistry is substantially repetitive with the addition of each monomer unit to the growing oligomer. Also, methods will be available for analyzing the composition and/or sequence of the oligomeric affinity group.

Alternatively, the affinity group may comprise small synthetic organic molecules having a molecular weight of at least about 200, and not more than about 5,000, generally ranging from about 250 to 2,000.

Generally, the oligomers employed will have at least 3 monomeric units and usually fewer than 20 monomeric units. The oligomers usually have at least 4, but fewer than 12 monomeric units and more preferably fewer than 10 monomeric units. Most preferably, the oligomers have between 4 to 8 monomeric units. The monomer units comprising an oligomeric affinity group may be naturally occurring or synthetic, generally being from about 2 to 30 carbon atoms, usually from about 2 to 18 carbon atoms and preferably from about 2 to 12 carbon atoms.

For affinity groups in the library, one or more monomeric units of the oligomer may remain constant, to reduce the overall complexity of the combinatorial library.

If the affinity group is an oligopeptide, the amino acid monomers may be naturally occurring or synthetic. Conveniently, the naturally occurring L-α-amino acids will be used, although the D-enantiomers may also be employed.

While the amino acid monomers of the oligomer may be any one of the 20 naturally occurring amino acids in either the L- or D-configuration, the amino acids employed will preferentially be free of reactive functionalities, particularly reactive functionalities which would react with the reactive functionality (R) of the affinity label compound. Therefore, the amino acids used in the invention will usually be free of reactive amino groups, frequently also being free of thiol groups. Of particular interest are such amino acids as alanine (A), glycine (G), proline (P), valine (V), serine (S), phenylalanine (F), isoleucine (I) and leucine (L) or uncharged polar amino acids like methionine (M). Other suitable amino acids include glutamate, aspartate and

aromatic amino acids, such as histidine (H), tryptophan (W), tyrosine (Y), and arginine (R).

Amino acid monomers of the oligomeric affinity group may also be synthetic. Thus, any unnatural or substituted amino acids of from 4 to 30, usually from 4 to 20, carbon atoms may be employed. Of particular interest are the synthetic amino acids β-alanine and γ-aminobutyrate or functional group protected amino acids such as O-methyl-substituted threonine (T), serine (S), tyrosine (Y), or the like. Various synthetic peptides are disclosed in Stewart, et al., *Solid Phase Peptide Synthesis*, W.H. Freeman, Co., San Francisco (1969); Bodanszky, et al., *Peptide Synthesis*, John Wiley and Sons, Second Edition (1976); J. Meienhofer, *Hormonal Proteins and Peptides*, 2: 46, Academic Press (1983); Merrifield, *Adv. Enzymol.* 32: 221-96 (1969); Fields, et al., *Int. Peptide Protein Res.*, 35: 161-214 (1990) and U.S. Patent 4,244,946 for solid phase peptide synthesis and Schroder, et al., *The Peptides*, Vol. 1, Academic Press (N.Y.) (1965), each of which is hereby incorporated by reference.

Amino acids useful in the present invention may have the carboxyl group at a site other than the terminal carbon atom, the amino group at a site other than the α-position or may be substituted with groups other than oxy, thio, carboxy, amino or guanidino, e.g., cyano, nitro, halo, particularly fluorine, oxo, inorganic acyl groups, etc. Synthetic amino acids may also be monosubstituted on nitrogen as in peptoids, which are oligomers of *N*-substituted glycine residues. *N*-substituted amino acids which find use will have an *N*-substituent of from about 1 to 8 carbon atoms, usually 1 to 6 carbon atoms, which may be aliphatic, alicyclic, aromatic or heterocyclic, usually having not more than about 3 heteroatoms, which may include amino (tertiary or quaternary), oxy, thio, and the like.

Oligopeptides may be constructed by employing standard Merrifield solid phase synthetic methods, manually or by using an automated peptide synthesizer, standard protection chemistry (e.g., *t*-Boc or Fmoc chemistry) and resins (e.g., 4-methyl benzhydryl amine Rink Amide resin). Successive rounds of deprotection of the terminal amino group and coupling of amino acid monomers, followed by deprotection and cleavage of peptides from resins results in the synthesis of

oligopeptides of the desired sequence and length. Additionally, liquid phase peptide synthesis is well known in the art and may also be employed.

If the amino acid monomers employed are *N*-substituted glycine residues, monomers may incorporate *t*-butyl-based side chain and 9-fluorenylmethoxycarbonyl α-amine protection. (See, for example, Gordon, et al., J. of Medicinal Chemistry (1994) 37, 1387-1385, and references cited therein.) Controlled oligomerization of the *N*-substituted monomers may be performed manually and/or robotically with *in situ* activation by either benzotriazol-1-yloxytris (pyrrolidino)-phosphonium hexafluorophosphate or bromotris (pyrrolidino) phosphonium hexafluorophosphate. Additional steps may follow standard automated peptide synthesis protocols using α-(9-fluorenylmethoxycarbonyl) amino acids.

General Connecting Groups (C_a and C_b)

Compositions of the invention may include one or more connecting components between the therapeutic or *in vitro* or *in vivo* diagnostic agent and the affinity group. The connecting groups may provide for synthetic convenience, particular physical characteristics of the total composition, e.g., water solubility, reduced non-specific binding, lipid solubility, and introduction of markers to allow for better identification of the molecule, e.g. dyes, chromophors, fluorophores and radiolabelled elements inclusive of C_{14} and H_3.

For the most part, the connector(s) will be bifunctional, about 1-20 atoms in length, which atoms may be carbon, nitrogen, oxygen, sulfur, phosphorus, and the like. The connector(s) may be alkylene groups, generally of from 2-16, more usually of from 1-25, carbon atoms, polyoxyalkylene groups, where the alkylene groups will be of 2-3 carbon atoms, and having from 1-8, more usually of from about 1-6, units, an amino acid, including alpha and omega amino acids, or oligopeptide having from 1-8, usually 1-6, amino acids, where the amino acids may be polar or non-polar, charged or uncharged, aliphatic, alicyclic, aromatic or heterocyclic, naturally occurring or synthetic. The connector(s) may also have the structure of an affinity group as described above, thereby providing additional binding affinity at the target site.

General Entities (E)

In general, the entity may be any moiety that may be linked to the reactive group and will covalently bond to the target molecule. Thus, an entity according to the invention can be any of a wide variety of biologically active or non-biologically active compounds that can bond to targets in a complex mixture. Examples of entities that may be particularly useful in the present invention include *in vitro* and *in vivo* diagnostic agents and therapeutic agents.

A. In Vitro Diagnostic Agents

In vitro diagnostic agents find use in *in vitro* diagnostic analysis. Such *in vitro* agents include biotin, fluorophors (such as fluorescein), chromophors, radiolabelled probes and chemiluminescent agents and the like, as recognized by those of skill in the art.

B. In Vivo Diagnostic Agents

In another aspect of the invention, the entity may be a compound which allows the diagnostic visualization of specific sites or compartments within the body by employing such diagnostic techniques as positron emission tomography (PET), computerized tomography (CT), single photon emission computerized tomography (SPECT), magnetic resonance imaging (MRI), nuclear magnetic imaging (NMI), fluoroscopy, ultrasound, etc. For such applications, the entity may comprise one or more contrast agents, radioisotopes of such elements as iodine (I), including ^{123}I, ^{125}I, ^{131}I, etc., barium (Ba), gadolinium (Gd), technetium (Tc), including ^{99}Tc, phosphorus (P), including ^{31}P, iron (Fe), manganese (Mn), thallium (Tl), chromium (Cr), including ^{51}Cr, carbon (C), including ^{11}C, or the like, fluorescently labeled compounds, etc. Such entities are also useful for identifying the presence of particular target sites in a mixture, to label molecules in a mixture, and the like. Various examples are found in U.S. Application 08/588,912 assigned to the assignee of this application and hereby incorporated by reference.

C. Therapeutic Agents.

A variety of suitable therapeutic agents are disclosed in U.S. Patent application Nos. 08/702,127 and 08/477,900, each of which is hereby incorporated by reference. In this regard, entities are generally less that 10,000 in molecular weight. Entities are such that they perform the desired function in the environment in which they become covalently bonded. For the most part, that environment will be an aqueous environment, usually serum or the interstitium. For use as drugs, the entity may be an agonist, an antagonist, a specific binding compound, an enzyme inhibitor (where the enzyme may be either soluble or membrane bound), a metal chelator, a factor supplement, a molecular scavenger, such as vitamin E, or the like. More specifically, the entity may include thrombin inhibitors, such as argatroban (for example see Application Number 08/674,315 which is hereby incorporated by reference), renin inhibitors, ACE inhibitors, inhibitors of the coagulation and complement cascade, serine proteases, $\alpha_v\beta_3$ antagonists, GPIIb/IIIa antagonists, CRF antagonists, or the like.

General Library Synthesis

Combinatorial libraries of affinity groups may be prepared in accordance with conventional ways for producing combinatorial libraries, particularly using a solid support and adding the monomeric components in a stepwise manner. See, for example, U.S. Patents 4,883,092; 5,010,175; 5,182,366 and 5,270,170 and PCT applications WO 92/00091; WO 92/09300; WO 93/06121; WO 93/20242; WO 94/06451; WO 94/06291 and WO 95/28640, as exemplary of a much larger literature of techniques. Preferably, the synthetic chemistry is substantially repetitive with the addition of each monomer unit to the growing oligomer.

For purposes of this disclosure, the subject affinity label compounds will be synthesized by conventional methods, although those of ordinary skill will appreciate that other methods may be used. Synthesizers are commercially available for synthesizing oligonucleotides and oligopeptides, as reflected in the references cited above. In addition, various conventional chemistries may be employed. Depending upon the nature of the functional group, the connector and the entity, synthetic strategies will be devised which allow for synthesis of the affinity marker library molecules at reasonable yields and without the formation of complex mixtures. As

those of skill will appreciate, the particular synthetic strategy will be determined empirically and on a case by case basis. Methods for combining various compounds are well known in the literature and can be employed with advantage. Where precursors to the entity are known, particularly prodrugs for drugs, the precursor or prodrug will frequently indicate the site for attachment and the nature of a linking group. Where prodrugs are not available, the physiologically active molecule may be modified stepwise at different sites, and the activity of the resulting compound determined.

General Library Screening

Initially, one will have a combinatorial library of compounds having varying oligomeric binding determinants to be screened for their affinity to a target molecule or site. Generally, the compounds will represent at least 50 different affinity groups, frequently 100 different affinity groups, usually at least about 500 different affinity groups, preferably at least about 1000 different affinity groups, and may be 10,000 or more different affinity groups, although usually the libraries will have 5,000 or fewer different affinity groups. The library may have greater proportions of one compound over other compounds, but desirably, the relative concentrations will differ by less than about 50%, preferably less than about 25%. For the screening process, the libraries may be divided into smaller units, which will generally range from about 5 to 1,000, frequently from about 5 to 500, usually from about 10 to 500 moieties and more usually from about 10 to 250. During this initial screening process, the library will include a first member of a binding pair (for instance, biotin for the binding pair biotin-avidin), and the first member will be screened with the second member of the binding pair

For identification of affinity marker members of the library having particular affinity for a particular target site relative to other members of the library, as indicated previously, depending upon the size of the library, some or all of the members of the library may be combined with the pure target compound in an appropriate reaction medium. The composition of the medium will vary widely, depending upon the nature of the target compound and the environment in which the subject affinity label

compounds will be used for bonding to the target compound. For the most part, the media will be polar, particularly aqueous, and may be buffered or otherwise modified to more closely mimic the ultimate environment in which the subject compounds will be used. The concentrations of the target compound and the library members may vary widely, usually being determined empirically, so as to optimize the differentiation between the various members of the library. Generally, for screening purposes, concentrations of the target compound will be in the range of about 0.05 to 5 μM, preferably in the range of about 0.1 to 1.0 μM, while concentrations of the library will vary in the range of about 10 to 150 μM, preferably in the range of from about 50 to 100 μM and more preferably in the range of from about 75 to 85 μM.

The temperature of the reaction may vary broadly as long as it is compatible with the reactants' and media stability. The temperature is thus frequently room temperature or the ambient temperature in which the subject compounds will be used. To the extent that the subject compositions will be used physiologically, the temperature will generally be between about 34-40 °C, more usually about 37 °C.

The determination of affinity for the target exhibited by the various affinity marker members of the library may be made by determining the composition of the liberated affinity groups at a single time point or a plurality of time points after the reaction is initiated. For the most part, those affinity groups which are liberated the earliest after the reaction is initiated are those which exhibit the greater binding affinity for that target site. Usually, the reaction will be allowed to proceed until there is a sufficient population of liberated affinity groups to allow for their ready determination and differentiation. Preferably, the reaction will be interrupted by the addition of a quenching chemical solution, thus allowing the fastest bonding members only to add to the target protein. In general, reactions terminated by quenching at short reaction times are preferred. Due to the large influence of the quenching solution on the behavior of the screening process, the time when the quenching solution is added will be studied as a separate screening parameter. Quenching times of 1 and 15 minutes will be studied. The affinity label leaving groups may be analyzed by any convenient means, including mass spectrometry, gel electrophoresis, chromatography, e.g., HPLC, TLC, or the like, where, if appropriate, the separated components may

then be sequenced. Where a plurality of aliquots of the library used, those sequences demonstrating preferred affinities may then be combined in a subsequent determination for direct comparison.

General Library Screening: Experimental Procedures

The screening of libraries that contain an entity which becomes covalently bonded to the target protein as a result of the affinity labeling reaction described herein may be conducted in two sequential steps and serves as a specific example of a general method for detecting the existence of a covalent adduct. First, a selected target protein reacts with the affinity labeling library or portion thereof. This step is typically conducted at room temperature, although not exclusively so, in the wells of polypropylene 96-well plates. Specified wells contain the target protein typically present at a concentration between 0.1 and 1μM in a buffered solution of appropriate composition and pH for maintaining the native biological structure and function of the target protein. Labeling reactions are initiated by adding a solution of affinity labeling library members in DMSO to give typical concentrations of total library and DMSO of 80 μM and 2% (volume/volume), respectively. The labeling reactions are terminated at a specified time, as short as 5 seconds or longer than 1 hour, by the addition of a suitable quench reagent (i.e., hydroxylamine for quenching thioesters) that combines with unreacted library members to form other species that do not react with the target protein. As controls, specified wells of the plate contain reaction mixtures that measure the amount of non-covalent binding of the entity to the target protein. These control reactions are conducted by first mixing the library with the quench reagent for a time sufficient to give forms of the library that do not react with the target protein and then adding the target protein.

Second is the detection of the entity that covalently bonds to the target protein, using standard methodologies known in the art, but in a novel time dependent process. For example, if the entity that covalently bonds to the target protein is biotin (or any first binding member of a binding pair), Enzyme-Linked-Immunoassays (ELISA's) can be employed to detect covalent bonding. Briefly, in these assays, polystyrene 96-well plates are coated with an antibody that specifically binds the target protein and

modified forms thereof. A portion of each reaction mixture is then transferred to the corresponding wells of the antibody-coated plate, and the binding of target protein to antibody allowed to ensue for approximately 2 hours. The plate is then emptied and 10 times filled with and emptied of phosphate buffered saline (PBS, 10 mM Pi, pH 7.4, 137 mM NaCl, 2.7 mM KCl) to remove non-covalently bound biotin from the well. A solution containing the enzyme conjugate avidin-horse-radish peroxidase (or any second member of a binding pair) may then be added to each washed well, and the binding of avidin of the conjugate to biotin of the modified target molecule, already bound to the antibody of the plate, is complete after 30 minutes. The plate may then be washed as described above; hydrogen peroxide and orthophenyl diamine are added as substrates of the peroxidase enzyme to give a visual measure of the amount of conjugate present in each well; conjugate amount is proportional to the amount of modified target protein in the well. The optical densities of each well are measured, and the values obtained are recorded in a computer spreadsheet and analyzed.

While the ELISA method of screening described above is specific for biotin-containing proteins, the method is, in principle, applicable for measurement of any affinity labeling library that results in the formation of a detectable adduct with any target biomolecule that can be sequestered at the surface of a 96-well plate.

General *In Vivo* or *In Vitro* Use of an Identified Affinity Group

Once a particular affinity group is identified, the group may be utilized *in vivo* for delivery of therapeutic or diagnostic agents or *in vitro* through coupling to an *in vitro* diagnostic agent for analysis of molecules to which the affinity group associates.

A. *In vitro*

The subject invention thus provides an efficient approach to identifying affinity label compounds that can be used to direct a specific entity to a target site. The subject invention allows for marking specific targets, particularly proteins, for a wide variety of purposes. Where complex mixtures are involved, such as blood, by using the present invention, one can enhance bonding to a specific component in the

blood, more particularly, a specific site on the target. In this way, one may achieve specific targeting to mobile components, such as blood cells, e.g., erythrocytes and platelets, proteins, such as immunoglobulins, serum albumin, transferrin, and the like. In mixtures in culture, one may specifically inhibit an enzyme target, so as to prevent enzymatic interference with the culture. For example, one may provide for specific bonding to RNases to prevent degradation of RNA. One may inhibit specific hydrolases, oxidoreductases, or the like. Where one wishes to mark a particular target, one can utilize fluorescent, radioactive, or other entity, which may be detected.

B. *In vivo*

When administered physiologically, the targets will usually be proteins, either individually, as aggregates with the same or different proteins or as surface membrane proteins.

The subject affinity label compounds, when administered physiologically, will usually be administered as a bolus, but may be introduced slowly over time by transfusion using metered flow, or the like. Alternatively, although less preferable, blood may be removed from the host, contacted with the affinity label compound *ex vivo*, and returned to the host. The affinity label compounds will be administered in a physiologically acceptable medium, e.g., deionized water, phosphate buffered saline, saline, mannitol, aqueous glucose, alcohol, vegetable oil or the like. Usually, a single injection will be employed, although more than one injection may be used, if desired. The affinity label compounds may be administered by any convenient means, including syringe, catheter or the like. The particular manner of administration will vary depending upon the amount to be administered, whether a single or sequential bolus, or continuous administration, or the like. Administration will be intravascular, where the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g., intravenously, peripheral or central vein. The intent is that the compound administered be effectively distributed in the vascular system so as to be able to react with target molecules therein.

The dosage of the affinity label compound will depend upon the entity employed and will, therefore, depend on the adverse effects of the entity, if any, the time necessary to reduce the unbound concentration of the affinity label compound present in the vascular system, the indication being sought, the sensitivity of the compound to destruction by vascular components, the route of administration and the like. As necessary, the dosage of affinity label compound may be determined empirically, initially using a small multiple of the dosage normally administered, and as greater experience is obtained, enhancing the dosage. Dosages will generally be in the range of 1 ng/kg to 10 mg/kg, usually being determined empirically in accordance with known ways, as provided for in preclinical and clinical studies.

The following examples are offered by way of illustration, and not by way of limitation.

Examples

In this section, we first describe the construction of S-, O-, and N-linked affinity label libraries: examples 1, 2, and 3 respectively. We then show the results for screening of an S-linked library against HSA at the level of 81 compounds per well (example 4), 9 compounds per well (example 5), and 1 compound per well (example 6). The results of these experiments indicate that -F*I*YEE is the preferred affinity group for bonding to HSA. Next, results of experiments characterizing the bonding of biotin to HSA using a -F*I*YEE affinity group are shown. Specifically, example 7 describes methods and materials used for the biological experiments and in examples 8 through 14, we show the results of these experiments.

Example 1: Construction of S-linked Affinity Label Libraries

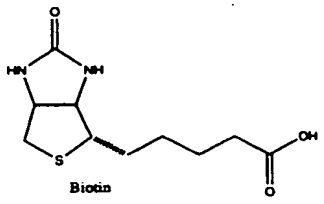
A representative affinity label library according to the invention and having a *p*-thiobenzoic acid residue as a reactive group and a thioester bond as a reactive functional group was designed and constructed. The structure of the library is as follows:

E-C_a-SPhCO-C_b-A.

An example of an S-linked library member is Biotin-SPhCO-O_1-O_2-X_1-X_2-B-NH_2 ,in which there is no C_a.

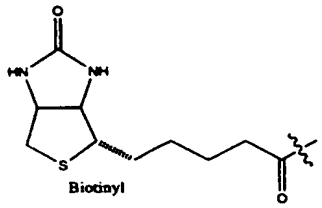
In the above described structure, X_1 and X_2 represent that all of the 9 selected L-amino acids appear in a well, O_1 represents that only one of the 9 selected L-amino acids appear in a well, O_2 represents that only one of the 9 selected D-amino acids appears in a well, and B represents that only one of the 9 selected L-amino acids appears in all 81 wells of a set. Biotin serves as the "entity," as described herein. The "SPhCO" represents a *p*-thiobenzoyl reactive group R between the biotin "entity" and an oligomeric affinity group (O_1-O_2-X_1-X_2-B-NH_2).

Biotin is represented by the following structure:



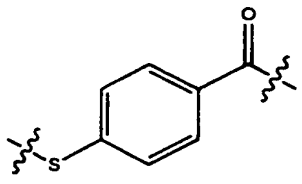
Biotin

Biotinyl is represented by the following structure:



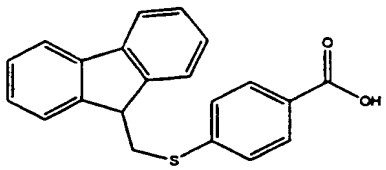
Biotinyl

Thiobenzoyl is represented by the following structure:



thiobenzoyl

S-9-Fluorenylmethyl *p*-thiobenzoic acid (Fm-S-Ph-CO_2H) is represented by the following structure:



Fm-SPh-CO2H

The precursor for Fm-S-Ph-CO_2H, 9-fluorenylmethyl chloride (Fm-Cl), was synthesized as follows. A solution of 25.0 g 9-fluorenylmethanol (127 mmol, Aldrich Chemical) was refluxed with 150 mL thionyl chloride for 30 minutes. The excess thionyl chloride was removed by distillation and the residue distilled at reduced pressure, then crystallized from ethanol two times to afford 19.2 g 9-fluorenylmethyl chloride (89.4 mmol, 70%) as a pale yellow solid.

Fm-S-Ph-CO_2H was prepared as follows: To a 0 °C suspension of 3.40 g 4-mercaptobenzoic acid (85%, Toronto Research Chemicals) (18.7 mmol) in 60 mL *N*, *N*-dimethylformamide (DMF) was added 4.00 g 9-fluorenylmethyl chloride (Fm-Cl) (Bodansky, M.; Bednarek, M. A. *Int. J. Pept. Protein Res.* **1982**, *20*, 434) synthesized as described above, (18.7 mmol) and 6.90 mL diisopropylethylamine (37.4 mmol). The reaction was allowed to slowly warm to RT and then stirred for 16 h. The cloudy yellow-brown solution was washed with hexanes (3 x 50 mL), diluted with 50 mL 1M HCl and extracted with ethylacetate (3 x 50 mL), washed with 50 mL sat. aq. NaCl,

dried with anhyd. $MgSO_4$, filtered, and concentrated via rotary evaporation to afford a yellow-brown oil. This oil was then dissolved in 20 mL MeOH, cooled to -20 °C and the resulting crystals were collected by filtration, washed with ice cold MeOH and dried to afford 5.59 g of Fm-S-Ph-CO_2H as a white solid (13.0 mmol, 70%).

The nine amino acids employed were carefully selected by considerations of the side chain functional groups and are listed in Table 1.

TABLE 1

Code	Amino Acid	Category	Starting Material
1	glutamic acid	acid	Fmoc-Glu(OtBu)-OH
2	glutamine	amide	Fmoc-Gln(Trt)-OH
3	arginine	base	Fmoc-Arg(Pmc)-OH
4	methionine	sulfide	Fmoc-Met-OH
5	serine	alcohol	Fmoc-Ser(tBu)-OH
6	tyrosine	phenol	Fmoc-Tyr(tBu)-OH
7	leucine	aliphatic	Fmoc-Leu-OH
8	phenylalanine	aromatic	Fmoc-Phe-OH
9	tryptophan	aromatic	Fmoc-Trp(Boc)-OH

The D-amino acids, O_2 , were introduced in order to prevent facile peptidyl cleavage *in vivo* and to maximize the affinity to the macromolecular targets.

In each set, there are 81 wells and in each well, there are 81 compounds while B is fixed. Such results in a library of 6561 total library members.

For example, when glutamic acid (E) was chosen to be the residue "B" in the above formula, the set 1 of combinatorial p-thiobenzoyl-containing affinity labeling libraries Biotin-SPhCO-O_1-O_2-X_1-X_2-E-NH_2 was constructed as follows:

The synthesis utilized Merrifield solid phase peptide synthesis methods and Fmoc chemistry. Rink amide MBHA resin was used to furnish amide at the C-terminus.

A schematic illustration outlining the construction of this set of libraries described above is shown in Figure 3.

In step (1) shown in Figure 3, Fmoc-Glu(OtBu)-OH was coupled to an identical amount of resin support in all 9 reaction vessels after removal of the Fmoc protecting group on Rink amide MBHA resin.

Removal of the Fmoc group was performed by treating with 20% piperidine in *N*-methylpyrrolidinone (NMP) twice, 2 minutes and 15 minutes, respectively at room temperature, followed by several NMP washes.

Coupling of the Fmoc-Glu(OtBu)-OH to the resin support was performed by adding to the resin in a reaction vessel with an *N,N*-dimethylformamide (DMF) solution of the amino acid (4 equivalents), o-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) (4 equivalents), 1-hydroxybenzotriazole (HOBt) (4 equivalents) and diisopropylethyl amine (DIEA) (8 equivalents). The reactions were allowed to proceed for 2 hours at room temperature. Completion of each reaction was monitored by a ninhydrin test to detect the presence of unreacted amino groups. The coupling reactions were repeated until the ninhydrin test for each reaction was negative. Once coupling was complete, as evidenced by a negative ninhydrin test, the peptide resin in each of the nine separate reactions was washed repeatedly with DMF.

Before the second L-amino acids were coupled to an identical amount of resin bearing the same Fmoc protected amino acid (i.e., Fmoc-Glu(OtBu)-OH) in all 9 reaction vessels), the Fmoc protecting group was removed by treating with 20% piperidine in NMP twice, 2 minutes and 15 minutes, respectively, at room temperature, followed by several NMP washes.

As shown in step (2) of Figure 3, each of the nine different Fmoc-protected L-amino acids shown in Table 1 were then individually linked to an identical amount of resin in all 9 reaction vessels. Coupling reactions were again carried out by adding to the resin in a reaction vessel with a DMF solution of the amino acid (4 equivalents),

HBTU (4 equivalents), HOBt (4 equivalents) and DIEA (8 equivalents). The reactions were allowed to proceed for 2 hours at room temperature. Completion of each reaction was monitored by a ninhydrin test. The coupling reactions were repeated until the ninhydrin test for each reaction was negative. Once coupling was complete as evidenced by negative ninhydrin test, the peptidyl resin in each of the nine separate reactions was washed with DMF.

The result of this second round of coupling is the generation of nine equimolar independent and distinct pools of dipeptides linked to resin support. As shown in Figure 3, since each of the nine independent and distinct pools contains one distinct dipeptide, there are now 9 distinct dipeptides generated.

As shown in step (3) of Figure 3, the resins from each of the nine independent and distinct pools generated in step (2) were mixed and then divided into identical pools. This gives rise to nine identical pools, each consisting of nine distinct dipeptides linked to the resin support.

Before the third round of coupling, the Fmoc protecting group was removed by treating with 20% piperidine in NMP twice, 2 minutes and 15 minutes, respectively, at room temperature, followed by several NMP washes.

As shown in step (4) of Figure 3, each of the nine different Fmoc-protected L-amino acids shown in Table 1 were individually linked to an identical pool of resin in all nine reaction vessels. Coupling reactions were carried out as described above.

As shown in step (5) of Figure 3, the resins from each of the nine independent and distinct pools generated in step (4) were mixed and then divided into identical pools, producing nine identical pools, each consisting of 81 distinct tripeptides linked to the resin support.

Before the fourth round of coupling, the Fmoc protecting group was removed by treating with 20% piperidine in NMP twice, 2 minutes and 15 minutes, respectively at room temperature, followed by several NMP washes.

As shown in step (6) of Figure 3, each of the nine different Fmoc-protected amino acids shown in Table 1 were linked to an identical pool of resin in all nine reaction vessels. In this round of coupling, unlike any of the other round of coupling,

amino acids in the D-configuration are employed. Coupling reactions were again carried out as described above.

This fourth round of coupling generates nine equimolar independent and distinct pools of tetrapeptides linked to resin support. As shown in Figure 3, since each of the nine independent and distinct pools contains 81 distinct tetrapeptides, there are now 729 distinct tetrapeptides generated.

As shown in step (7) of Figure 3, each of the nine independent and distinct pools of tetrapeptides generated in step (6) were each divided into a set of nine identical pools without mixing the pools before dividing, giving rise to nine sets of identical pools (81 pools total), each pool containing 81 tetrapeptides. The step of splitting the pools without mixing permits the fourth and fifth amino acids coupled to the resin to be defined. Before the fifth round of coupling, the Fmoc protecting group was removed by treating with 20% piperidine in NMP twice, 2 minutes and 15 minutes, respectively, at room temperature, followed by several NMP washes.

As shown in step (8) of Figure 3, each of the nine identical pools in each of the nine sets of pools generated in step (7) was then coupled by one of the nine Fmoc-protected amino acids shown in Table 1. Coupling reactions were again carried out as described above.

This fifth round of coupling generates eighty-one equimolar independent and distinct pools of pentapeptides linked to a resin support. As shown in Figure 3, since each of eighty-one independent and distinct pools contains eighty-one distinct pentapeptides, 6561 distinct pentapeptides have been generated.

As shown in step (9) of Figure 3, each of the eighty-one independent and distinct pools generated in step (8) was then coupled to S-9-fluorenylmethyl- *p*-thiobenzoic acid (Fm-S-Ph-CO_2H). Before the coupling of Fm-S-Ph-CO_2H, the Fmoc protecting group was removed by treating with 20% piperidine in NMP twice, 2 minutes and 15 minutes, respectively at room temperature, followed by several NMP washes.

Coupling reactions were carried out by adding to the resin in a reaction vessel with a DMF solution of S-9-fluorenylmethyl-*p*-thiobenzoic acid (4 equivalents), HBTU (4 equivalents), HOBt (4 equivalents) and DIEA (8 equivalents). The reactions

were allowed to proceed for 2 hours at room temperature. Completion of each reaction was monitored by a ninhydrin test. The coupling reactions were repeated until the ninhydrin test for each reaction was negative. Once coupling was complete as evidenced by a negative ninhydrin test, the peptidyl resin in each of the nine separate reactions was washed with DMF.

Before the last round of coupling, the fluorenylmethyl protecting group was removed by treating with 20% piperidine in NMP twice, 2 minutes and 10 minutes, respectively at room temperature, followed by NMP washes rapidly.

As shown in step (10) of Figure 3, each of eighty-one independent and distinct pools generated in step (8) was then coupled to biotin. Coupling reactions were carried out by adding to the resin in a reaction vessel with a DMF solution of biotin (5 equivalents), HBTU (5 equivalents), HOBt (5 equivalents) and DIEA (10 equivalents). The reactions were allowed to proceed for 2 hours at room temperature. The coupling reactions were repeated to ensure completion.

Finally, to complete the construction of the *p*-thiobenzoyl-containing affinity labeling libraries, as shown in step (11) of Figure 3, the completely constructed affinity labeling libraries were cleaved from the resin component and cleavage and purification were performed as follows.

The peptidyl resin mixtures in each 81 independent and distinct pools generated in step (10) were dried and then independently treated with trifluoroacetic acid (TFA)/H_2O (95/5, v/v) for 1.5 hours. The peptide/TFA solutions were then precipitated then washed with ether. Following ether precipitation, the peptide solutions were suspended in 0.045%TFA in water and lyophilized, to yield the completely constructed and dried *p*-thiobenzoyl-containing affinity labeling library.

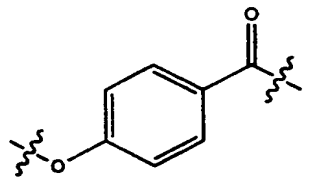
The final combinatorial *p*-thiobenzoic-containing affinity labeling library is substantially stable under normal storage conditions as determined by mass spectral analysis.

Example 2: Construction of O-Linked Affinity Labeling Libraries

In addition to S-linked libraries, O-linked libraries can be synthesized having a *p*-hydroxybenzoic acid as a reactive functional group. Individual solid phase peptide

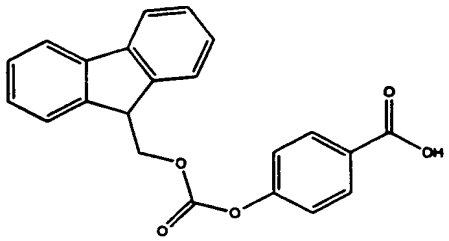
synthesis is performed on an Applied Biosystems 433A Peptide Synthesizer using FastMoc Chemistry. Multiple peptide syntheses are performed on a Gilson AMS 422 Multiple Peptide Synthesizer using Fmoc chemistry. Solid phase reactions were monitored by ninhydrin test for completion. Amino acids and Rink Amide MBHA resin were obtained from NovaBiochem. Preparative HPLC was performed on a 21.4 x 250 mm C_{18} reverse phase column using 5-60% B (0.045% TFA in H_2O and 0.045% TFA in CH_3CN) gradient elution. Liquid Chromatography/Mass Spectrometry results were obtained using electrospray ionization on a Perkin Elmer Sciex API300 Mass Spectrometer using a 1.0 x 250 mm C_{18} reverse phase protein/peptide column using 0-70% B (0.045% TFA in H_2O and 0.045% TFA in CH_3CN) gradient elution.

The structure of *p*-hydroxybenzoyl is as follows:



hydroxybenzoyl

The structure of Fmoc-OPh-CO_2H is as follows:



Fmoc-OPh-CO2H

Synthesis of 9 Individual Library Members

Synthesis was performed on the Gilson AMS 422 Multiple Peptide Synthesizer using 25 μmol Rink amide MBHA resin/reaction tube. Deprotection of the Rink amide MBHA resin and subsequent deprotections of the Fmoc amino acids were

carried out using 25% piperidine in *N,N*-dimethylformamide (DMF) (4 x 5 min each) followed by a DMF wash (6 times). Coupling of the amino acids, each dissolved in *N*-methylpyrrolidinone (NMP), was performed two using 300 μL of 0.68 M amino acid solution (8 eq) in *N*-methylpyrrolidinone (NMP), 440 μL of 0.45 M solution of *o*-benzotriazol-1-yl-*N, N, N', N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) (8 eq) in DMF, and 100 μL of 4 M *N*-methylmorpholine (NMM) (16 eq) in DMF were added and allowed to react for 1 h. Upon completion, dichloromethane (DCM) was added and reaction continued for 1 h. The reaction mixtures were then filtered and washed with DMF (6 times) and the next amino acid coupling was performed. The first coupling reaction was Glu, the second was one of nine different amino acids (Glu, Gln, Arg, Met, Ser, Tyr, Leu, Phe, and Trp), one amino acid/tube, the third coupling was Tyr, followed by D-Leu, then Phe.

The addition of the O-Ph group was performed in exactly the same way as an amino acid coupling. First, the last amino acid added was deprotected and washed, then 300 μL of 0.68 M Fmoc-O-Ph-CO_2H (8 eq) in DMF, 440 μL of a 0.45 M solution HBTU/ HOBt (8 eq) in DMF, and 100 μL of 4 M NMP (16 eq) in DMF were added and allowed to react for 1 h. Then, DCM was added and reaction continued for 1 h, followed by the removal of the Fmoc group with 25% piperidine in DMF.

Biotin was added by reacting 980 μL of a 128 mM biotin isobutyl carbonic anhydride (5 eq) solution (2 x 10 h each). The resins were then washed with DMF (6 times) and DCM (6 times).

Each of the peptidyl resin mixtures was then independently treated with 0.75 mL trifluoroacetic acid (TFA):H_2O:phenol (10:0.5:0.75, v/v/wt) (2 x 1 h each), washed with 0.75 mL TFA and 0.75 mL DCM. The combined filtrates were then concentrated via speed vac and products isolated by precipitation with dry-ice cold Et_2O (3 mL) followed by centrifugation. The resulting white solids were then washed with dry-ice cold Et_2O (2 x 3 mL each), then dissolved in 1.5 mL of 0.045% TFA in H_2O and 1.5 mL of 0.045% TFA in CH_3CN, allowed to stand at RT for 1 h then purified by reverse phase prep HPLC to afford the products, after lyophylization, as white solids.

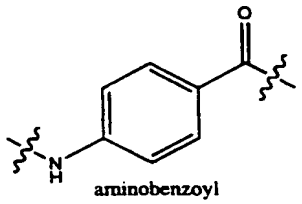
Synthesis of biotin isobutyl carbonic anhydride

In a 50-mL round-bottomed flask equipped with a magnetic stir bar and N_2 inlet adapter was dissolved 822 mg biotin (3.30 mmol) in 23.9 mL hot DMF. After cooling to room temperature, 1.38 mL triethylamine (9.90 mmol) was added followed immediately by 428 µL isobutylchloroformate (3.30 mmol). Immediately, a precipitate formed and the reaction was allowed to stir at RT for 10 min. The insoluble precipitate (TEA•HCl) was removed by filtration to afford the approximately 128 mM biotin isobutyl carbonic anhydride solution.

Example 3: Construction of N-Linked Affinity Labeling Libraries

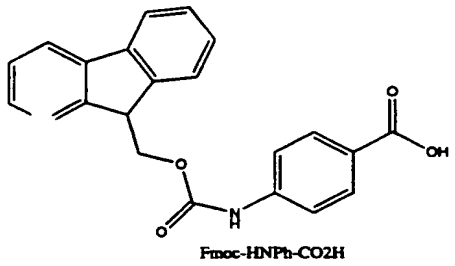
In addition to S-linked and O-linked libraries, N-linked libraries can be synthesized having a *p*-aminobenzoic acid as a functional group. Individual solid phase peptide syntheses can be performed on an Applied Biosystems 433A Peptide Synthesizer using FastMoc Chemistry. Multiple peptide syntheses can be performed on a Gilson AMS 422 Multiple Peptide Synthesizer using Fmoc chemistry. Solid phase reactions can be monitored by ninhydrin test for completion. Amino acids and Rink Amide MBHA resin can be obtained from NovaBiochem. Preparative HPLC can be performed on a 21.4 x 250mm C_{18} reverse phase column using 5-60% B (0.045% TFA in H_2O and 0.045% TFA in CH_3CN) gradient elution. Liquid Chromatography/Mass Spectrometry analysis can be obtained using electrospray ionization on a Perkin Elmer Sciex API300 Mass Spectrometer using a 1.0 x 250 mm C_{18} reverse phase protein/peptide column using 0-70% B (0.045% TFA in H_2O and 0.045% TFA in CH_3CN) gradient elution.

The structure of *p*-aminobenzoyl is as follows:



aminobenzoyl

The structure of Fmoc-HNPh-CO_2H is as follows:



Fmoc-HNPh-CO2H

Synthesis of 9 Individual Library Members

Synthesis can be performed on the Gilson AMS 422 Multiple Peptide Synthesizer using 25 μmol Rink amide MBHA resin/reaction tube. Deprotection of the Rink amide MBHA resin and subsequent deprotections of the Fmoc amino acids can be carried out using 25% piperidine in *N,N*-dimethylformamide (DMF) (4 x 5 min each) followed by a DMF wash (6 times). Coupling of the amino acids, each dissolved in *N*-methylpyrrolidinone (NMP), can be performed two using 300 μL of 0.68 M amino acid solution (8 eq) in NMP, 440 μL of 0.45 M solution of o-benzotriazol-1-yl-*N, N, N', N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) (8 eq) in DMF, and 100 μL of 4 M *N*-methylmorpholine (NMM) (16 eq) in DMF are then added and allowed to react for 1 h. Upon completion, dichloromethane (DCM) was added and the reaction continued for 1 h. The reaction mixtures are then filtered and washed with DMF (6 times) and the next amino acid coupling was performed. The first coupling reaction can be Glu, the second was one of nine different amino acids (Glu, Gln, Arg, Met, Ser, Tyr, Leu, Phe, and Trp), one amino acid/tube, the third coupling was Tyr, followed by D-Leu, then Phe.

The addition of the N-Ph group can be performed in exactly the same way as an amino acid coupling. First, the last amino acid added is deprotected and washed, then 300 μL of 0.68 M Fmoc-NH-Ph-CO_2H-S-Ph-CO_2H (8 eq) in DMF, 440 μL of 0.45 M solution HBTU/HOBt (8 eq) in DMF, and 100 μL of 4 M NMP (16 eq) in DMF are added and allowed to react for 1 h. Upon completion, DCM is added and reaction continued for 1 h, followed by the removal of the Fmoc group with 25% piperidine in DMF.

Biotin is added by reacting 980 μL of a 128 mM biotin carbonic anhydride (5 eq) solution (2 x 10 h each). The resins are then washed with DMF (6 times) and DCM (6 times).

Each of the peptidyl resin mixtures can then independently treated with 0.75 mL trifluoroacetic acid (TFA):H_2O:phenol (10:0.5:0.75, v/v/wt) (2 x 1 h each), washed with 0.75 mL TFA and 0.75 mL DCM. The combined filtrates are then concentrated via speed vac and products isolated by precipitation with dry-ice cold Et_2O (3 mL) followed by centrifugation. The resulting white solids are then washed with dry-ice cold Et_2O (2 x 3 mL each), then dissolved in 1.5 mL of 0.045% TFA in H_2O and 1.5 mL of 0.045% TFA in CH_3CN, allowed to stand at RT for 1 h then purified by reverse phase prep HPLC to afford the products, after lyophylization, as white solids.

Synthesis of Individual Compounds

Biotin Isobutylcarbonic Anhydride Solution

In a 10-mL round-bottomed flask equipped with a magnetic stir bar and N_2 inlet adapter was dissolved 300 mg of biotin (1.23 mmol) in 5 mL of hot DMF. This was allowed to cool to RT where 677 μL of triethylamine (4.86 mmol) was added followed immediately by 160 μL of isobutylchloroformate (1.23 mmol). Immediately, a precipitate formed and the reaction was allowed to stir at RT for 10 min. The insoluble precipitate (TEA•HCl) was removed by filtration and washed with 2-mL of DMF to afford 8 mL of the approximately 154 mM biotin carbonic anhydride solution containing approximately 453 mM TEA (3.63 mmol).

Synthesis of Biotin-OPh-CO-F*I*YEE-NH_2

The procedure followed for this synthesis is illustrated schematically in Figure 16. Synthesis of the fragment HO-Ph-CO-F*I*YEE-PS was synthesized using Applied Biosystems 433A peptide synthesizer using FastMoc chemistry (HBTU/HOBt/DIEA) with final deprotection on a 0.250 mmol scale using Fmoc-Rink amide MBHA resin (NovaBiochem) in 98% yield. The resulting fragment was then transferred to a 20-mL glass solid phase reaction vessel and washed with three 4-mL portions of DMF. This

was then shaken with 8 mL of an approximately 150 mM solution of biotin isobutylcarbonic anhydride solution (1.23 mmol) with approximately 453 mM TEA (3.63 mmol) for 70 h. This was then filtered and washed with six 4-mL portions of DMF and three 4-mL portions of DCM. The resin was then treated with two 4-mL portions of cleavage cocktail solution (10 mL TFA, 0.5 mL water and 0.75 g phenol) for 1 h each. The resin was then washed with 4 mL TFA and 4 mL DCM and the combined cleavage and washing filtrates were concentrated by rotary evaporation to 2 mL. The crude product was then isolated by precipitation with 30 mL dry-ice cold Et_2O followed by centrifugation. After decanting the supernatant, the resulting white solid was re-suspended in 30 mL dry-ice cold ether, centrifuged, and decanted two times. This was then dissolved in 10 mL of 0.045% TFA in CH_3CN and 10 mL of 0.045% TFA in water, allowed to stand at RT for 1 h, frozen and lyophilized to afford 219 mg of the crude product as a white solid. Analytical HPLC reveals product to be approximately 80% pure (contains approximately 10% HO-Ph-CO-F*I*YEE-NH2). This was then purified by preparative reverse-phase HPLC to afford 121 mg of biotin-OPh-CO-F*I*YEE-NH_2 as a white solid (0.115 mmol, 47%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.05 (br s, 2 H), 9.06 (br s, 1 H), 8.57 (d, J = 8.0 Hz, 1 H), 8.19-8.17 (m, 3 H), 7.84-7.81 (m, 3 H), 7.32 (d, J = 7.4 Hz, 1 H), 7.28 (br s, 1 H), 7.24 (t, J = 7.5 Hz, 2 H), 7.17 (d, J = 8.7 Hz, 2 H), 7.14 (d, J = 7.2 Hz, 1 H), 7.07 (br s, 1 H), 7.00 (m, 3 H), 6.58 (d, J = 8.4 Hz, 2 H), 6.44 (br s, 1 H), 6.35 (br s, 1 H), 4.73-4.70 (m, 1 H), 4.47-4.43 (m, 1 H), 4.33-4.30 (m, 1 H), 4.30-4.20 (m, 2 H), 4.20-4.14 (m, 2 H), 3.16-3.12 (m, 1 H), 3.04 (dd, J = 13.5, 5.0 Hz, 1 H), 2.97-2.93 (m, 2 H), 2.83 (dd, J = 12.5, 5.0 Hz, 1 H), 2.64-2.54 (m, 4 H), 2.30-2.24 (m, 2 H), 2.20 (t, J = 8.0 Hz, 2 H), 1.95-1.85 (m, 2 H), 1.85-1.76 (m, 1 H), 1.76-1.70 (m, 1 H), 1.70-1.60 (m, 3 H), 1.56-1.42 (m, 1 H), 1.42-1.35 (m, 2 H), 1.16-1.12 (m, 3 H), 0.71 (d, J = 6.0 Hz, 3 H), 0.69 (d, J = 6.0 Hz, 3 H). Anal. HPLC indicated product to be >95% pure with R_t = 48.34 min. ESI-MS *m/z* for $C_{51}H_{65}N_8O_{14}S$ (MH^+), calcd 1045.4 found 1045.8.

_N_-Tritylglycine Isobutylcarbonic Anhydride Solution

In a 10-mL round-bottomed flask equipped with a magnetic stir bar and N_2 inlet adapter was dissolved 333 mg _N_-trityl glycine (Aldrich) (1.05 mmol) in 2 mL

DCM. To this was added 558 µL triethylamine (4.86 mmol) was added followed immediately by 130 µL isobutylchloroformate (1.23 mmol). Immediately, a precipitate formed and the reaction was allowed to stir at RT for 5 min. The insoluble precipitate (TEA•HCl) was removed by filtration and washed with 2 mL DCM to afford approximately 5 mL of the approximately 200mM *N*-tritylglycine isobutylcarbonic anhydride solution containing approximately 600 mM TEA (3.00 mmol).

Synthesis of Biotin-Gly-OPh-CO-F*I*YEE-NH_2

The procedure for this synthesis is illustrated schematically in Figure 17. Synthesis of the fragment HO-Ph-CO-F*I*YEE-PS was synthesized using Applied Biosystems 433A peptide synthesizer using FastMoc chemistry (HBTU/HOBt/DIEA) with final deprotection on a 0.250 mmol scale using Fmoc-Rink amide MBHA resin (NovaBiochem) in 98% yield. A 354 mg portion of the resulting fragment was then transferred to an 8-mL solid phase reaction vessel and washed with three 4-mL portions of DCM. This was then shaken with 5 mL of an approximately 200 mM solution of *N*-tritylglycine isobutylcarbonic anhydride solution (1.23 mmol) with approximately 600 mM TEA (3.63 mmol) for 24 h. This was then filtered and washed with six 2-mL portions of DCM, then treated with five 2-mL portions of (100:5:5) DCM/TFA/TIS (dichloromethane/trifluoroacetic acid/triisopropylsilane) for 2 min each and washed with six 2-mL portions of DCM, three 2-mL portions of DMF, 2 mL of 2 M DIEA in NMP and three 2-mL portions of DMF to afford a pale tan resin. This was then shaken with a solution of 122 mg biotin (0.500 mmol) in 1.10 mL of 0.45 M HBTU/HOBt in DMF, 250 µL of 2 M DIEA in NMP, and 1.10 mL DMF (heat was required for dissolution) for 1 h. The resin was then filtered and washed with six 2-mL portions of DMF and three 2-mL portions of DCM. The resin was then treated with two 2-mL portions of cleavage cocktail solution (10 mL TFA, 0.5 mL water and 0.75 g phenol) for 1.5 h each. The resin was then washed with 2 mL TFA and 2 mL DCM and the combined cleavage and washing filtrates concentrated by rotary evaporation to 2 mL. The crude product was then isolated by precipitation with 40 mL dry-ice cold Et_2O followed by centrifugation. After decanting the supernatant, the

resulting white solid was re-suspended in 40 mL dry-ice cold ether, centrifuged, and decanted two times. This was then dissolved in 6 mL of 0.045% TFA in CH_3CN and 6 mL of 0.045% TFA in water, allowed to stand at RT for 1 h, frozen and lyophilized to afford the crude product as a white solid. This was then purified by preparative reverse-phase HPLC to afford 29.4 mg of biotin-Gly-OPh-CO-F*l*YEE-NH_2 as a white solid (0.027 mmol, 21%). Anal. HPLC indicated product to be >95% pure with R_t = 45.43 min. ESI-MS *m/z* for $C_{53}H_{68}N_9O_{15}S$ (MH^+), calcd 1102.5, found 1103.0.

LC-Biotin Isobutylcarbonic Anhydride Solution

In a 10-mL round-bottomed flask equipped with a magnetic stir bar and N_2 inlet adapter was dissolved 108 mg of *N*-(+)-biotin-6-aminocaproic acid (0.302 mmol) in 6 mL of hot DMF. This was allowed to cool to RT where 167 µL of triethylamine (1.20 mmol) was added followed immediately by 39.2 µL of isobutylchloroformate (0.302 mmol). The reaction was then allowed to stir at RT for 10 min to afford approximately 6.2 mL of the approximately 50 mM LC-biotin isobutylcarbonic anhydride solution containing approximately 145 mM TEA (0.898 mmol).

Synthesis of LC-Biotin-OPh-CO-F*l*YEE-NH_2

The procedure followed for this synthesis is illustrated schematically in Figure 18. Synthesis of the fragment HO-Ph-CO-F*l*YEE-PS (PS is polystyrene) was synthesized using Applied Biosystems 433A peptide synthesizer using FastMoc chemistry (HBTU/HOBt/DIEA) with final deprotection on a 0.250 mmol scale using Fmoc-Rink amide MBHA resin (NovaBiochem) in 98% yield. A 0.120 mmol portion of the resulting fragment was then transferred to an 8-mL solid phase reaction vessel and washed with three 4-mL portions of DMF. This was then shaken with approximately 6.2 mL of approximately 50 mM solution of LC-biotin isobutylcarbonic anhydride solution (0.302 mmol) with approximately 145 mM TEA (0.898 mmol) for 21 h. This was then filtered and washed with six 2-mL portions of DMF and three 4-mL portions of DCM. The resin was then treated with two 1.5-mL portions of cleavage cocktail solution (10 mL TFA and 0.5 mL water) for 1.25 h each. The resin was then washed with 2 mL TFA and 2 mL DCM and the combined

cleavage and washing filtrates were concentrated by rotary evaporation to 1.5 mL. The crude product was then isolated by precipitation with 13 mL dry-ice cold Et_2O followed by centrifugation. After decanting the supernatant, the resulting white solid was re-suspended in 13 mL dry-ice cold ether, centrifuged, and decanted two times, then dried to afford 98.1 mg of a white solid. Analytical HPLC reveals product to be approximately 52% pure (contains approximately 48% HO-Ph-CO-F*l*YEE-NH_2). This was then dissolved in 4 mL of 0.045% TFA in CH_3CN and 4 mL of 0.045% TFA in water and purified by preparative reverse-phase HPLC to afford 47.2 mg of LC-biotin-OPh-CO-F*l*YEE-NH_2 as a white solid (0.041 mmol, 34%). Anal. HPLC indicated product to be >95% pure with R_t = 48.08 min. ESI-MS *m/z* for $C_{57}H_{76}N_9O_{15}S$ (MH^+), calcd 1158.5, found 1158.8.

_N_-Tritylglycine 4-Nitrophenylcarbonic Anhydride Solution

In a 10-mL round-bottomed flask equipped with a magnetic stir was dissolved 86.4 mg DMAP (*N,N*-dimethylaminopyridine) (0.708 mmol) in 1 mL EtOAc and 3 mL DCM. To this was added a solution of 36.2 mg 4-nitrophenylchloroformate (0.180 mmol) in 1 mL EtOAc, followed by a solution of 56.2 mg *N*-trityl glycine (Aldrich) (0.177 mmol) in 1 mL DCM. The reaction was then allowed to stir at RT for 15 min. The reaction was then concentrated *in vacuo*, then taken up in 1 mL DMF to afford the approximately 177 mM solution of *N*-tritylglycine 4-nitrophenylcarbonic anhydride containing approximately 531 mM DMAP.

Synthesis of Argatroban-AEA_3-βAla-Gly-OPh-CO-F*l*YEE-NH_2•2TFA

The procedure followed for this synthesis is illustrated schematically in Figure 19. A 0.035 mmol portion of the HO-Ph-CO-F*l*YEE-PS fragment was then transferred to a 4-mL solid phase reaction vessel and washed with three 1-mL portions of DMF. This was then shaken with approximately 1.0 mL of approximately 177 mM solution *N*-tritylglycine 4-nitrophenylcarbonic anhydride solution (0.177 mmol) with approximately 531 mM DMAP (0.531 mmol) for 2 h. This was then filtered and washed with six 2-mL portions of DMF and six 2-mL portions of DCM. The trityl group was then removed by vortexing a solution of 5% TFA/5% TIS in DCM (4 x 1

mL) for 1 min each. The resin was then washed with six 1-mL portions of DCM, six 1-mL portions of DMF, 1 mL of 2 M DIEA in NMP, and three 1-mL portions of DMF to afford a tan resin (the resulting resin showed a positive ninhydrin test). The resin was then treated with a solution of 38.0 mg of a 70:30 mixture of argatroban-AEA_3-βAla-OH: argatroban-AEA_3-βAla-NHS ester dissolved in a solution of 393 μL of 0.45 M HBTU/HOBt, 393 μL DMF, and 88.5 μL of 2 M DIEA in NMP for 1.25 h. The resin was washed with six 1-ml portions of DMF and six 1-mL portions of DCM to afford a tan resin (the resulting resin showed a negative ninhydrin test). The resin was then treated with two 1-mL portions of cleavage cocktail solution (10 mL TFA, 0.5 mL water and 0.5 mL TIS) for 1 h each. The combined cleavage filtrates were concentrated by rotary evaporation to 0.5 mL and crude product was then isolated by precipitation with 13 mL dry-ice cold Et_2O followed by centrifugation. After decanting the supernatant, the resulting white solid was re-suspended in 13 mL dry-ice cold ether, centrifuged, and decanted two times. This was then dissolved in 2 mL of 0.045% TFA in CH_3CN and 3 mL of 0.045% TFA in water, allowed to stand at RT for 1 h, frozen then lyophilized to afford 29.7 mg of a white solid. This was then dissolved in 4 mL of 0.045% TFA in CH_3CN and 4 mL of 0.045% TFA in water and purified by preparative reverse-phase HPLC to afford 6.7 mg of argatroban-AEA_3-βAla-Gly-OPh-CO-F*l*YEE-NH_2 • 2 TFA as a white solid (0.003 mmol, 10%). Anal. HPLC indicated product to be >80% pure with R_t = 54.77 min. ESI-MS *m/z* for $C_{81}H_{113}N_{17}O_{24}S$ (MH^+), calcd 1739.8, found MH^{2+} 871.1.

Synthesis of Fluorescein-thiourea-AEA_3-Gly-OPh-CO-F*l*YEE-NH_2

The procedure followed for this synthesis is illustrated schematically in Figure 20. A 0.035 mmol portion of the HO-Ph-CO-F*l*YEE-PS fragment was then transferred to a 4-mL solid phase reaction vessel and washed with three 1-mL portions of DMF. This was then shaken with approximately 1.0 mL of approximately 177 mM solution *N*-tritylglycine 4-nitrophenylcarbonic anhydride solution (0.177 mmol) with approximately 531 mM DMAP (0.531 mmol) for 2 h. This was then filtered and washed with six 2-mL portions of DMF and six 2-mL portions of DCM. The trityl group was then removed by vortexing a solution of 5% TFA/5% TIS in DCM (4 x 1

mL) for 1 min each. The resin was then washed with six 1-mL portions of DCM, six 1-mL portions of DMF, 1 mL of 2 M DIEA in NMP, and three 1-mL portions of DMF to afford a tan resin (the resulting resin showed a positive ninhydrin test). The resin was then shaken with a solution of 64.0 mg *N*-trityl aminoethoxyacetic acid (Trt-AEA-OH) (0.177 mmol) dissolved in a solution of 393 μL of a 0.45 M HBTU/HOBt, 393 μL of DMF, and 88.5 μL of 2 M DIEA in NMP for 1 h. The resin was washed with six 1-ml portions of DMF and six 1-mL portions of DCM to afford a tan resin (the resulting resin showed a negative ninhydrin test). The trityl group was then removed by vortexing a solution of 5% TFA/5% triisopropylsilane (TIS) in DCM (4 x 1 mL) for 1 min each, then washed with six 1-mL portions of DCM, six 1-mL portions of DMF, 1 mL of 2 M DIEA in NMP, and three 1-mL portions of DMF to afford a tan resin (the resulting resin showed a positive ninhydrin test). The tan resin was then shaken with a solution of 50.3 mg fluorescein isothiocyanate isomer I (FITC) (0.116 mmol) and 11 μL TEA (0.079 mmol) for 10 min. The resin was filtered and washed with six 1-mL portions of DMF and six 1-mL portions of DCM to afford a tan resin (the resulting resin showed a negative ninhydrin test). The resin was then treated with two 1-mL portions of cleavage cocktail solution (10 mL TFA, 0.5 mL water and 0.5 mL TIS) for 1 h each. The combined cleavage filtrates were concentrated by rotary evaporation to 0.5 mL and crude product was then isolated by precipitation with 13 mL dry-ice cold Et_2O followed by centrifugation. After decanting the supernatant, the resulting white solid was re-suspended in 13 mL dry-ice cold ether, centrifuged, and decanted two times. This was then dissolved in 2 mL of 0.045% TFA in CH_3CN and 3 mL of 0.045% TFA in water and purified by preparative reverse-phase HPLC to afford 6.4 mg fluorescein-thiourea-AEA-Gly-OPh-CO-F*l*YEE-NH_2 as a yellow-orange solid (0.005 mmol, 13%). Anal. HPLC indicated product to be >95% pure with R_t = 58.14 min. ESI-MS *m/z* for $C_{68}H_{70}N_9O_{20}S$ (MH^+), calcd 1364.4, found 1366.3, MH^{2+} 683.9.

Example 4a: HSA Screening of Library at level of 81 Compounds Per Well with a 1 minute quenching time

The S-linked library described in Example 1 was constructed and screened against HSA using the general experimental procedures described in the "General Library Screening" Section above. Using a 1 minute quenching time, the screening procedures were first carried out at the level of 81 compounds per well with 81 distinct wells. At this level of screening, (see Fig. 4) each well contains a mixture of compounds of the formula Biotin-S-Ph-C(O)-O_1-O_2-X_1-X_2-B-NH_2, with X_1 and X_2 fixed for each well and each well containing a mixture of the 81 combinations of possible O_1 and O_2 amino acids. B is fixed as glutamic acid. The amino acid residue at position O_2 is an L-amino acid; the amino acid residues at all other positions are D-amino acids. Quenching time was fixed at 1 minute.

A rapid quench (1 minute) destroys the slow unreacted library members, thus limiting nonspecific bonding due to reaction of the library members with other nucleophiles that compete with the same reactive functional group. Fast quenching is advantageous since it facilitates identification of a lead affinity group in spite of the limited amount of biotin added. ELISA assays were used and the optical density results shown in Table II were recorded. Table II shows sort values calculated as (ΔO.D.-2xS.D./(background O.D.) and represented in Figure 4.

TABLE II

	d-amino acid at position O_2								
l-amino acid at position O_1	Glu	Gln	Arg	Met	Ser	Tyr	Leu	Phe	Trp
Glu	1.3	0.8	1.2	0.9	1.0	1.3	0.4	1.1	1.4
Gln	2.0	0.9	1.4	1.2	1.4	1.3	0.7	1.2	1.1
Arg	1.0	0.6	2.2	1.0	1.0	1.5	1.1	1.2	1.3
Met	1.0	1.4	1.3	1.3	1.2	1.3	1.0	1.3	1.2
Ser	0.8	1.0	1.0	0.8	1.2	1.3	1.3	1.5	0.6
Tyr	1.3	1.2	1.4	1.1	1.0	0.8	1.7	1.4	1.5
Leu	1.2	1.0	1.2	1.3	1.4	0.3	1.1	1.4	1.4
Phe	1.4	0.8	1.4	1.4	1.2	0.7	3.5	1.2	1.5

	d-amino acid at position O_2								
l-amino acid at position O_1	Glu	Gln	Arg	Met	Ser	Tyr	Leu	Phe	Trp
Trp	1.2	1.4	1.3	1.1	1.5	1.0	0.7	1.5	1.5

From these results, it appears that molecules of the formula Biotin-S-Ph-C(O)-F-*l* -X_1-X_2-E-NH_2 show the highest measured optical density. The optical density (OD) is approximately proportional to the amount of biotin added at a site on a given target. An elevated OD can thus be explained as an elevated amount of biotinylation for a given well. The driving force behind the increased rate of biotinylation is explained in terms of the enhanced specificity brought by some of the most efficient affinity groups. As a result, an elevated OD for a given well may be the result of an enhanced specificity of addition for some of the members in that well. Using a one minute quench screening, biotin-S-Ph-C(O)-F-*l* -X_1-X_2-E-NH_2 shows high specificity for HSA based on optical density reading.

Example 4b: HSA Screening of Library at level of 81 Compounds Per Well with a 15 minute quenching time

The S-linked library described in Example 1 was constructed and screened against HSA using the general experimental procedures described in the "General Library Screening" section above. Using a 15 minute quenching time, the screening procedures were first carried out at the level of 81 compounds per well with 81 distinct wells. At this level of screening, (see Fig. 5) each well contains a mixture of compounds of the formula biotin-S-Ph-C(O)-O_1-O_2-X_1-X_2-B-NH_2, with X_1 and X_2 fixed for each well and each well containing a mixture of the 81 combinations of possible O_1 and O_2 amino acids. B is fixed as glutamic acid. The amino acid residue at position O_2 is an L-amino acid; the amino acid residues at all other positions are D-amino acids. Table III shows sort values calculated as (ΔO.D.-2xS.D./(background O.D.).and represented in Figure 5.

Table III

	d-amino acid at position O_2								
l-amino acid at position O_1	Glu	Gln	Arg	Met	Ser	Tyr	Leu	Phe	Trp
Glu	3.3	3.6	3.6	1.2	2.3	3.6	3.2	6.1	11.3
Gln	5.6	4.6	3.0	2.1	2.1	3.7	5.8	4.2	4.0
Arg	3.5	3.2	9.4	2.3	3.0	3.9	3.9	3.3	3.4
Met	2.8	3.5	4.3	2.3	4.1	3.2	4.1	3.5	3.4
Ser	3.6	4.0	2.9	2.3	3.7	2.7	4.0	4.3	5.3
Tyr	3.9	3.5	2.8	2.6	5.4	6.3	4.9	5.8	4.9
Leu	3.9	3.1	3.2	2.6	4.5	3.3	2.4	4.2	4.6
Phe	4.0	3.7	3.1	2.8	4.3	6.0	4.4	4.1	6.1
Trp	9.8	5.1	3.1	3.2	8.8	9.0	8.2	5.1	10.9

Using a 15 minute quenching time, biotin-S-Ph-C(O)-W-W -X_1-X_2-E-NH_2 shows high specificity for HSA based on the readout of the optical density measured for each well.

Example 5: HSA Screening of Library at level of 9 Compounds Per Well

Each particular set of candidate affinity groups, once identified, must be rescreened or deconvoluted. ELISA assays were carried out at the level of 9 compounds per well and 9 wells where the compounds have the formula biotin-S-Ph-C(O)-F-*l* -X_1-X_2-E-NH_2 with X_1 fixed for each well and each well containing the 9 possible X_2 amino acid residues. Construction of these compounds per well libraries is described below.

Figures 6a and 6b show optical density measurements from screening of the 9 wells. These results show that molecules of the formula Biotin-S-Ph-C(O)-F-*l* -Y-X_2-E-NH_2 show enhanced affinity for HSA.

Example 6: Biological Methods and Materials

Fraction V HSA (Calbiochem) was suspended in PBS (Sigma) at 15 or 600 μM and stored at -20 °C before use. Human plasma was obtained from Calbiochem, human serum from Sigma, and pig, dog, and human whole blood were obtained from healthy donors at Notre Dame Hospital in Montréal. The plasma was extracted by centrifugation of whole blood for 4 min at 500x g. D-biotin (Sigma) and NHS-LC-biotin (Pierce) were dissolved in DMSO (Sigma) at the required stock concentrations. Biotin-OPh-CO-F*l*YEE-NH_2, HO-Ph-CO- F*l*YEE -NH_2 , biotin-OPh-CO-NH_2 were dissolved in DMSO at the required stock concentrations.

Biotinylation of Fraction V human serum albumin, human serum and whole blood

In order to determine the specificity of F*l*YEE, either 100 or 600 μM of biotin-OPh-CO- F*l*YEE -NH_2 or biotin-OPh-CO-NH_2 was added to individual samples of whole blood, plasma or serum. The reaction samples were allowed to incubate for 1h at RT or 37 °C. For the whole blood sample, the plasma was extracted from whole blood at the end of reaction. Additional pig or dog plasma and fraction V HSA (600 μM final concentration) samples were prepared by mixing with 100 μM (final concentration) of biotin-OPh-CO- F*l*YEE -NH_2 or biotin-OPh-CO-NH_2 and allowed to incubate for 1h at RT or 37 °C. These additional samples were diluted 1/60 in PBS and analyzed directly on SDS-PAGE, immunoblot, ELISA, or frozen at -20 °C until use. As positive controls, 50 μM of NHS-LC-biotin (Pierce) and 100 μM of D-biotin were used. Protein assay was performed using BCA kit (Pierce).

Proteins were separated by SDS-PAGE under non-reducing conditions (Laemmli, 1970), transferred to 0.1 μM-pore nitrocellulose sheets (Schleicher and Schuell) and probed with peroxidase labeled streptavidin (Jackson Immuno Research). The biotinylated proteins were then revealed by an ECL detection kit (Pharmacia).

Competition ELISA test

A solution of 100 μL rabbit polyclonal anti-human serum albumin (Boehringer) diluted at 1/5000 in PBS was coated overnight at 4 °C onto ELISA plates (Nunc). These plates were then saturated with 5% BSA (Sigma) in PBS for 2 h at RT.

For the competition test, varying concentrations of HO-Ph-CO- F*I*YEE -NH_2, 44 μM biotin-OPh-CO-F*I*YEE-NH_2, and 15 μM of fraction V HSA were incubated for 1 h at 37 °C. To assess the ability of biotin-OPh-CO-F*I*YEE-NH_2 to displace HO-Ph-CO-F*I*YEE -NH_2, varying concentrations of HO-Ph-CO- F*I*YEE -NH_2 were incubated with HSA for 1 h at 37 °C, then 44 μM of biotin-OPh-CO-F*I*YEE-NH_2 was added and allowed to incubate at 37 °C for 1 h. The reaction mixtures were then centrifuged through 30 kDa Centricon tubes (Sarthorious) to a volume of 50 μL, diluted with 500 μL of PBS, then re-concentrated to 50 μL. This process was then repeated and transferred to the ELISA plate previously coated with polyclonal anti-human serum albumin (procedure described above). These plates were then rinsed with PBS-Tween 20 (0.05%) three times, then with PBS. Peroxidase-labeled streptavidin (1:500, v/v, in PBS containing 0.05% BSA) was then added to the plates, allowed to incubate for 20 min at RT, rinsed with PBS-Tween 20 (0.05%) three times, and then with PBS. These rinsed plates were then treated with 100 μL of 0.5 mg/mL solution of o-phenylene diethylamine (Sigma) in a 50 mM (pH 5) Na_2HPO_4/citrate buffer for 6 min at RT, then 50 μL of 2N H_2SO_4 was added to quench the reaction. The plates were then read on a SpectraMax 250 at λ 490 nm.

Kinetic study by ELISA capture

A solution of 100 μL of human plasma was treated with 100 μM of NHS-LC-biotin, biotin-BMCC (Pierce), biotin-OPh-CO-F*I*YEE-NH_2 or biotin-OPh-CO-NH_2. The samples were then allowed to incubate at RT for 0, 5, 10, 15, 30 and 60 min, when 5 μL of each sample was diluted with 295 μL PBS and kept at -20 °C before use. The samples were then diluted to 2 μg/mL using PBS and transferred to an ELISA plate previously coated with polyclonal anti-human serum albumin (procedure described above). The plates were then rinsed with PBS-Tween 20 (0.05%) three times, and then with PBS. Goat polyclonal anti-biotin (Pierce) (1:200, v/v, in PBS containing 0.05% BSA) was then added to the plates, allowed to incubate for 1 h at RT, rinsed with PBS-Tween 20 (0.05%) three times, and then with PBS. The plates were then treated with 100 μL of peroxidase-conjugated rabbit anti-goat IgG (Jackson Immuno Research) diluted 1/100,000 with PBS for 20 min at RT. The plates were

then rinsed with PBS-Tween 20 (0.05%) three times, and then with PBS. The plates were then treated with a 0.5 mg/mL solution of o-phenylene diethylamine (Sigma) in a 50 mM (pH 5) Na_2HPO_4/citrate buffer for 6 min at RT, then 50 µL of 2N H_2SO_4 was added to quench the reaction. The plates were then read on a SpectraMax 250 at λ 490 nm.

Example 7: Biological Results--Specificity of biotin-O-Ph-C(O)-F*I*YEE-NH_2 is Species Dependent

Whole blood was incubated with biotin ester for 1 h at room temperature, then plasma was extracted by centrifugation. A 5 µg sample was separated by 8% SDS-PAGE and transferred onto blot. The immunoblot was blocked with 5% skim milk (Nestlé) and then incubated with peroxidase labeled-streptavidin diluted at 1/5000 in PBS-BSA 0.5% for 20 min at RT. An ECL kit was used to detect peroxidase labeled-streptavidin on Hyperfilm ECL (Amersham), exposed for 1 min.

Example 8: Biological Results--Non-specificity of biotin-OPh-CO-NH_2: in absence of " F*I*YEE " peptide, the biotinylated compound loses specificity

Figure 7 shows an immunoblot in which human serum and plasma samples in lanes 1, 2 and 3 were labeled with 100 µM biotin-OPh-CO-NH_2 and samples in lanes 4, 5 and 6 with 600 µM biotin-OPh-CO-NH_2 for 1 h at 37 °C. A 5 µg portion of each sample was separated by 8% SDS-PAGE and transferred onto blot. The immunoblot was blocked with 5% skim milk (Nestlé) and then incubated with peroxidase labeled-streptavidin diluted at 1/5000 in PBS-BSA 0.5% for 20 min at RT. An ECL kit was used to detect peroxidase labeled-streptavidin on Hyperfilm ECL (Amersham), exposed for 1 min. Lanes 1 and 4 were fresh human plasma, lanes 2 and 5 were commercial human serum and lanes 3 and 6 were commercial human plasma.

Example 9: Biological Results--Competition of biotin-OPh-CO-F*I*YEE-NH_2 vs. HO-Ph-CO- F*I*YEE -NH_2

Figure 8 plots optical density as a function of the concentration of HO-Ph-CO-F*I*YEE -NH_2. In these experiments, solutions of 0 to 100 molar excess HO-Ph-CO-

F*l*YEE -NH_2 and 44 μM of biotin-OPh-CO-F*l*YEE-NH_2 were incubated with 15 μM of fraction V HSA for 1 h at 37 °C. The HSA was captured on an ELISA plate initially coated with polyclonal anti-HSA, treated with peroxidase labeled streptavidin and OD read at 490 nm using SpectraMax 250.

Example 10: Biological Results--Ability of biotin-OPh-CO-F*l*YEE-NH_2 to displace HO-Ph-CO- F*l*YEE -NH_2 on HSA

Figure 9 shows a plot of optical density as a function of the concentration of HO-Ph-CO-F*l*YEE-NH_2. In these experiments, solutions of 0 to 50 molar excess HO-Ph-CO-F*l*YEE-NH_2 and 15 μM fraction V HSA were incubated for 1 h at RT when 44 μM of biotin-OPh-CO-F*l*YEE-NH_2, was added and reaction incubated for 1 h at 37 °C. The HSA was captured on an ELISA plate initially coated with polyclonal anti-HSA, treated with peroxidase labeled streptavidin and OD read at 490 nm using SpectraMax 250.

Example 11: Biological Results--Rate of the reactivity of biotin-OPh-CO-F*l*YEE -NH_2 in plasma

Figure 9 shows the results of a kinetic study by ELISA capture experiment in which human plasma was treated with 100 μM biotin-OPh-CO-F*l*YEE-NH_2, biotin-OPh-CO-NH_2, NHS-LC-biotin or biotin-BMCC for 1 h at RT. At each time mentioned in the figure, 2 μg/mL of each sample was captured on an ELISA plate initially coated with polyclonal anti-HSA, treated with goat polyclonal anti-biotin, followed by peroxidase conjugated rabbit anti-goat IgG and OD read at 490 nm using SpectraMax 250.

Example 12: Biological Results--Kinetic Studies using HPLC

Rate determination was performed on a Rainin Dynamax HPLC system equipped with a 4.6 x 250 mm Microsorb 300Å C18 reverse phase column and UV detector at λ 214 and 245 nm running a gradient elution of 5-60% B (A: 0.045% TFA in H_2O and B: 0.045% TFA in CH_3CN) over 60 min at 0.5 mL/min. Each sample was prepared by mixing the appropriate amount of a stock solution of biotin-OPh-CO-

F*I*YEE -NH_2 or biotin-OPh-CO-NH_2 in either 600 μM fraction V HSA or commercial human plasma and injecting 20 μL of this solution onto the column using an autosampler at various time intervals. The chromatograms were each integrated using identical data parameters and resulting calculated areas under each peak converted to concentrations and plotted against time.

Figure 11 plots the rate of disappearance of biotin-OPh-CO-NH_2 vs. time and the rate of appearance of the leaving group, HO-Ph-CO-NH_2, vs. time.

Figure 12 plots the rate of appearance of the leaving group, HO-Ph-CO-F*I*YEE-NH_2, vs. time (seconds) as a result of the reaction of 100 μM biotin-OPh-CO-F*I*YEE-NH_2 in commercial human plasma. Due to overlapping peaks in the HPLC chromatogram, the rate of disappearance of biotin-OPh-CO-F*I*YEE-NH_2 cannot be determined.

Figure 13 shows a plot of the rate of hydrolysis of biotin-OPh-CO-F*I*YEE-NH_2 vs. time (seconds) and the rate of appearance of the leaving group, HO-Ph-CO-F*I*YEE-NH_2, vs. time (seconds).

Figure 14 shows a plot of the rate of appearance of the leaving group, HO-Ph-CO-F*I*YEE-NH_2, vs. time (seconds). Due to overlapping peaks in the HPLC chromatogram, the rate of disappearance of biotin-OPh-CO-F*I*YEE-NH_2 cannot be determined.

Figure 15 shows HPLC chromatographs of tryptic digest of HSA (top) and HSA:LC-biotin (bottom) where LC-biotin was introduced using LC- biotin-OPh-CO-FIYEE- NH_2. LC-biotin is biotin-NH-$(CH_2)_5$-C(O)-.

Discussion of Biological Results

Selectivity

Biotin-OPh-CO-F*I*YEE-NH_2 is selective to HSA. Immunoblots show the specificity of biotin-OPh-CO-F*I*YEE-NH_2 for human serum albumin (HSA) and not for pig or dog albumin in samples of whole blood.

Specificity

The specificity with which biotin-OPh-CO-F*I*YEE-NH_2 reacts with a specific lysine residue on HSA was determined by mass spectral analysis of a tryptic digest of LC-biotin conjugated to HSA by LC-biotin-OPh-CO-F*I*YEE-NH_2 (Figure 15). Using LC-biotin-OPh-CO-F*I*YEE-NH_2 instead of biotin-OPh-CO-F*I*YEE-NH_2 facilitated a larger mass separation in the mass spectrum and identification of HSA from HSA:LC-biotin. The differences in the two chromatograms are not immediately obvious, however; careful analysis of these chromatograms reveals two new peaks (Figure 15, arrows indicate the new peaks at 78 and 85 minutes). Mass spectral analysis of the new peak at 78 min showed a new peptide fragment with a mass of 2887.6 which corresponds to a peptide sequence of ^{501}EFNAETFNAETFTFHADIBTLSELYSKER^{521} (where B = carboxymethyl cysteine) containing only one lysine residue where LC-biotin is attached, Lys_{519}. Mass spectral analysis of the new peak at 85 min showed a new peptide fragment with a mass of 5903.4, corresponding to a peptide sequence of: ^{476}BBTESLVNRRPBFSALEVDETYVPK^{500}EFNAETFNAETFTFHADIBTLSEKER^{521} (where B = carboxymethyl cysteine), which contains two lysine residues (K_{500} and K_{519}). This mass accounts only for the addition of one LC-biotin and since the fragment at 78 min contains only one Lys residue and LC-biotin is attached to it, and this peptide sequence contains the previous sequence, the only lysine residue in this sequence where biotin could be attached is Lys_{519}.

Because biotin-OPh-CO-F*I*YEE-NH_2 labels HSA selectively, is specific (Figure 15) and biotin-OPh-CO-NH_2 does not label selectively (Figure 7), it appears that biotin-OPh-CO-NH_2 will not label specifically and that the driving force to specificity is at least in part determined by the affinity portion of the molecule, the pentapeptide F*I*YEE-NH_2.

Reaction Kinetics

Prima facie evidence for the affinity of the F*I*YEE binding determinants for a specific site on the surface of HAS and its ability to direct pendant groups to such sites is supported by the results in Figures 8 and 9. The reaction of biotin-OPh-CO-F*I*YEE-NH_2 with HSA not only depends on concentration, but also on the amount of

leaving group (HO-Ph-CO-F*I*YEE-NH_2) present. Figures 8 and 9 suggest competition for the active site on HSA between biotin-OPh-CO-F*I*YEE-NH_2 and the leaving group HO-Ph-CO-F*I*YEE-NH_2, which competition could effect the rate of binding and subsequent bonding of biotin to HSA. As shown in Figure 8, direct competition of 0-100 molar excess of HO-Ph-CO-F*I*YEE-NH_2 and biotin-OPh-CO-F*I*YEE-NH_2 with 15 μM HSA indicates that the excess HO-Ph-CO-F*I*YEE-NH_2 occupies a specific binding site on HSA, thus preventing the binding and subsequent bonding of biotin-OPh-CO-F*I*YEE-NH_2. As Figure 9 further illustrates, prior incubation of HO-Ph-CO-F*I*YEE-NH_2 with HSA prevents binding of biotin-OPh-CO-F*I*YEE-NH_2. This competition is observed only at the relatively higher concentrations of HO-Ph-CO-F*I*YEE-NH_2; between 1 to 5 molar excess of HO-Ph-CO-F*I*YEE-NH_2, competition has a very minor effect on the rate of reaction.

The influence of the affinity portion of the molecule (F*I*YEE-NH_2) on the rate of reaction is clearly seen by comparison of 100 μM biotin-OPh-CO-NH_2 and 600 μM HSA in PBS (Figure 11) with 100 μM biotin-OPh-CO-F*I*YEE-NH_2 and 600 μM HSA in PBS (Figure 14). The half-reaction for biotin-OPh-CO-NH_2 is about 330 min whereas that of biotin-OPh-CO-F*I*YEE-NH_2 is about 125 min. Under these reaction conditions, the rate of hydrolysis of the phenol ester is very slow (Figure 13) and has a half-life of about 7.2 days. Not only does the affinity portion of the molecule greatly influence the rate of reaction; the reaction medium or the form of albumin has a tremendous effect on the rate of reaction. The half-reaction of with 100 μM biotin-OPh-CO-F*I*YEE-NH_2 in commercial human plasma is about 6 min. This dramatic change in rate is most likely due to conformational differences of HSA when in PBS and in plasma. This very rapid rate of reaction when in plasma is truly remarkable and is compelling evidence that an in vivo administration of a drug-OPh-CO-F*I*YEE-NH_2 molecule could be used as a possible therapeutic or diagnostic delivery agent.

Figures 10 and 12 show the kinetic of addition of biotin-OPh-CO-F*I*YEE-NH_2. Figure 10 shows the accelerated rate of biotin addition of biotin-OPh-CO-F*I*YEE-NH_2 when compared to biotin-OPh-CO-NH_2 (on the base line). As shown on Figure 12, after only five minutes, more than half of a 100 μM biotin-OPh-CO-F*I*YEE-NH_2 has reacted with its protein target (in this case commercial human plasma).

We claim:

1. A compound according to the formula E-C_a-R-C_b-A, wherein E is a therapeutic or diagnostic agent, R is a reactive group, C_a and C_b are connector groups between E and R and between R and A, respectively, and A is a group having an affinity for human serum albumin, wherein affinity group A comprises a sequence of amino acid residues -O_1-O_2-X_1-X_2-B in which the amino acid residues are independently selected from the group of all twenty naturally occurring amino acids.

2. A compound according to claim 1, wherein affinity group A comprises the sequence -O_1-O_2-X_1-X_2-B- wherein:

amino acid residue O_1 is selected from the group consisting of phenylalanine, arginine, glutamine, tyrosine and tryptophan;

amino acid residue O_2 is selected from the group consisting of leucine, arginine, glutamic acid, tryptophan and phenylalanine;

amino acid residue X_1 is selected from the group consisting of phenylalanine, tryptophan, methionine and tyrosine;

amino acid residue X_2 is selected from the group consisting of serine, arginine and glutamic acid; and

amino acid residue B is selected from the group consisting of serine, arginine and glutamic acid.

3. A compound according to claim 2, wherein at least one of the amino acid residues is a D-amino acid and at least one is an L-amino acid.

4. A compound according to claim 2, wherein one of the five amino acid residues is an L amino acid residue and the other four amino acid residues are D amino acid residues.

5. A compound according to claim 3, wherein the L-amino acid residue is selected from the group consisting of the amino acid residue O_2, the amino acid residue X_1, and the amino acid residue X_2.

6. A compound according to claim 2, wherein one of the five amino acid residues is a D-amino acid residue and the other four amino acid residues are L-amino acid residues.

7. A compound according to claim 6, wherein the D-amino acid residue is selected from the group consisting of the amino acid residue O_2, amino acid residue X_1, and amino acid residue X_2.

8. A compound according to claim 7, wherein the D-amino acid residue is the amino acid residue O_2.

9. A compound according to claim 2, wherein O_1 is phenylalanine and O_2 is leucine.

10. A compound according to claim 2, wherein O_1 is arginine and O_2 is arginine.

11. A compound according to claim 2, wherein O_1 is glutamine and O_2 is glutamic acid.

12. A compound according to claim 2, wherein O_1 is glutamic acid and O_2 is tryptophan.

13. A compound according to claim 2, wherein O_1 is tryptophan and O_2 is tryptophan.

14. A compound according to claim 2, wherein O_1 is tryptophan and O_2 is glutamic acid.

15. A compound according to claim 2, wherein X_1 is tyrosine.

16. A compound according to claim 2, wherein X_2 is glutamic acid.

17. A compound according to claim 2, wherein B is glutamic acid.

18. A compound according to claim 2, wherein O_1 is phenylalanine, O_2 is D-leucine, X_1 is tyrosine, X_2 is glutamic acid, and B is glutamic acid.

19. A compound according to claim 2, wherein the amino acid residue B is a C-terminal amino acid residue.

20. A compound according to claim 19, wherein the affinity group comprises the amino acid sequence $-O_1-O_2-X_1-X_2-B-NH_2$.

21. A compound according to claim 2, wherein the compound further includes a reactive group attached to the affinity group, and wherein the reactive group includes a functional group selected from the group consisting of carboxy, phosphoryl, alkyl esters, thioesters, phosphoesters, ortho esters, imidates, mixed anhydrides and disulphides.

22. A compound according to claim 21, wherein the reactive group is bonded directly to the O_1 amino acid residue in the affinity group.

23. A compound according to claim 22, wherein the reactive group is bonded to the O_1 amino acid residue by an amide linkage.

24. A compound according to claim 21, wherein the reactive group has the formula $-X-R_1-C(O)-$, where C(O) is an alpha carboxyl, R_1 includes a substituted or unsubstituted aromatic group and X is selected from the group consisting of S, O and N.

25. A compound according to claim 24, wherein X is bonded directly to an aromatic carbon atom in R_1.

26. A compound according to claim 24, wherein R_1 is unsubstituted phenyl.

27. A compound according to claim 26, wherein the –X- and –C(O)- substituents are bonded to the unsubstituted phenyl is a para configuration.

28. A compound according to claim 24, wherein R_1 is phenyl substituted with one or more groups selected from the group consisting of a halogen, NO_2, SO_2NR_2, SO_3R, SO_2NH_2, SO_2NHF, NR_3^+, CF_3, CCl_3, CBr_3, $C\equiv N$, SO_3H, CO_2H, CO_2R, CHO, $CORNH_2$, NHR, NR_2, OH, $NHCOCH_3$, NHCOR, OCH_3, OR, CH_3, CH_2CH_3 and RC_6H_5.

29. A compound according to claim 24, wherein the reactive moiety is bonded directly to the O_1 residue via the carboxyl carbon.

30. A compound according to claim 21, further comprising a first connecting group connecting the reactive group and the affinity group.

31. A compound according to claim 30, wherein the first connecting group is bonded to the reactive group via an ester, thioester, amide, sulfonate ester or sulfonamide linkage.

32. A compound according to claim 30, wherein the first connecting group is bonded to the O_1 amino acid residue in the affinity group via an ester, thioester, amide, sulfonamide, urea, thiourea or carbamate linkage.

33. A compound according to claim 30, wherein the first connecting group includes a backbone chain of between about 1 and about 25 atoms.

34. A compound according to claim 33, wherein the first connecting group includes a backbone chain of between about 2 and about 16 carbon atoms.

35. A compound according to claim 30, wherein the first connecting group includes an unsaturated carbon atom backbone chain of between about 1 and about 25 atoms.

36. A compound according to claim 21, further including an entity bonded to the reactive group.

37. A compound according to claim 36, wherein the entity is a therapeutic or diagnostic agent.

38. A compound according to claim 36, wherein the entity is bonded directly to the reactive group by a linkage selected from the group consisting of an amide linkage, an ester linkage, a thioester linkage and a sulfonate ester linkage.

39. A compound according to claim 38, wherein the entity is bonded to the reactive group by an ester or thioester linkage.

40. A compound according to claim 36, further comprising a second connecting group connecting the entity to the reactive group.

41. A compound according to claim 40, wherein the second connecting group is bonded to the entity by an ester, thioester, amide, sulfonate ester or sulfonamide linkage.

42. A compound according to claim 40, wherein the second connecting group is bonded to the reactive group by an ester, thioester, amide or sulfonate ester linkage.

43. A compound according to claim 40, wherein the second connecting group includes a backbone chain of between about 1 and about 25 atoms.

44. A compound according to claim 43, wherein the second connecting group includes a backbone chain of between about 2 and about 16 carbon atoms.

45. A compound according to claim 40, wherein the second connecting group includes an unsaturated carbon atom backbone chain of between about 1 and about 25 atoms.

46. A compound according to claim 36, wherein the entity comprises a biotinyl group.

47. A compound according to claim 46, wherein the biotinyl group is bonded directly to the reactive group by an ester, thioester or amide linkage.

48. A compound according to claim 46, wherein the reactive group has the formula –X-Ph-C(O)-, and where X is oxygen, sulfur or nitrogen.

49. A compound according to claim 48, wherein the –X- and –C(O)- substituents on the Ph group are bonded is a para configuration.

50. A compound according to claim 47, further comprising a second connecting group connecting the biotin group to the reactive group.

51. A compound according to claim 50, wherein the second connecting group is bonded to the biotin group by an amide linkage.

52. A compound according to claim 50, wherein the second connecting group is $-NH-(CH_2)_n-C(O)-$, where n is an integer between 1 and 25.

53. A compound according to claim 52, wherein the second connecting group is $-NH-(CH_2)_5-C(O)-$.

54. A compound according to claim 52, wherein the second connecting group is –NH-CH_2-C(O)-.

55. A compound selected from the group consisting of biotin-S-Ph-C(O)-F*l*YEE-NH_2, biotin-OPh-C(O)-F*l*YEE-NH_2, LC-biotin-S-Ph-C(O)-F*l*YEE-NH_2 , biotin-Gly-OPh-C(O)-F*l*YEE-NH_2, fluorescein-Gly-OPh-F*l*YEE-NH_2, LC-biotin-OPh-C(O)-F*l*YEE-NH_2, argatroban-AEA_3-βAla-Gly-OPh-C(O)-F*l*YEE-NH_2, and fluorescein-thiourea-AEA_3-Gly-OPh-C(O)-F*l*YEE-NH_2.

56. A method for screening for the affinity of a compound for human serum albumin, comprising the steps of:

a) immobilizing the albumin on a test substrate;

b) incubating the compound with the albumin under conditions that support covalent interaction between the compound and the albumin;

c) quenching the interaction between the albumin and the compound; and

d) assaying for activity of the albumin, wherein the compound is of the formula E-C_a-R-C_b-A, wherein E is a therapeutic or diagnostic agent, R is a reactive group, C_a and C_b are connector groups between E and R and between R and A, respectively, and A is a group having an affinity for albumin, wherein affinity group A comprises a sequence of amino acid residues in which the amino acid residues are independently selected from the group of all twenty naturally occurring amino acids.

57. .....A method according to claim 56 wherein the entity E is selected from the group consisting of biotin, fluorescein and argatroban.

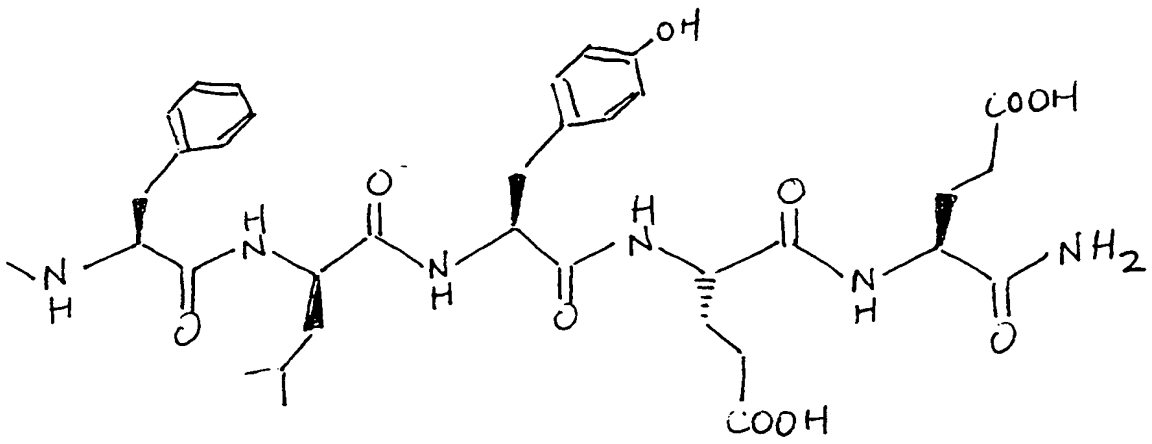


FIG. 1

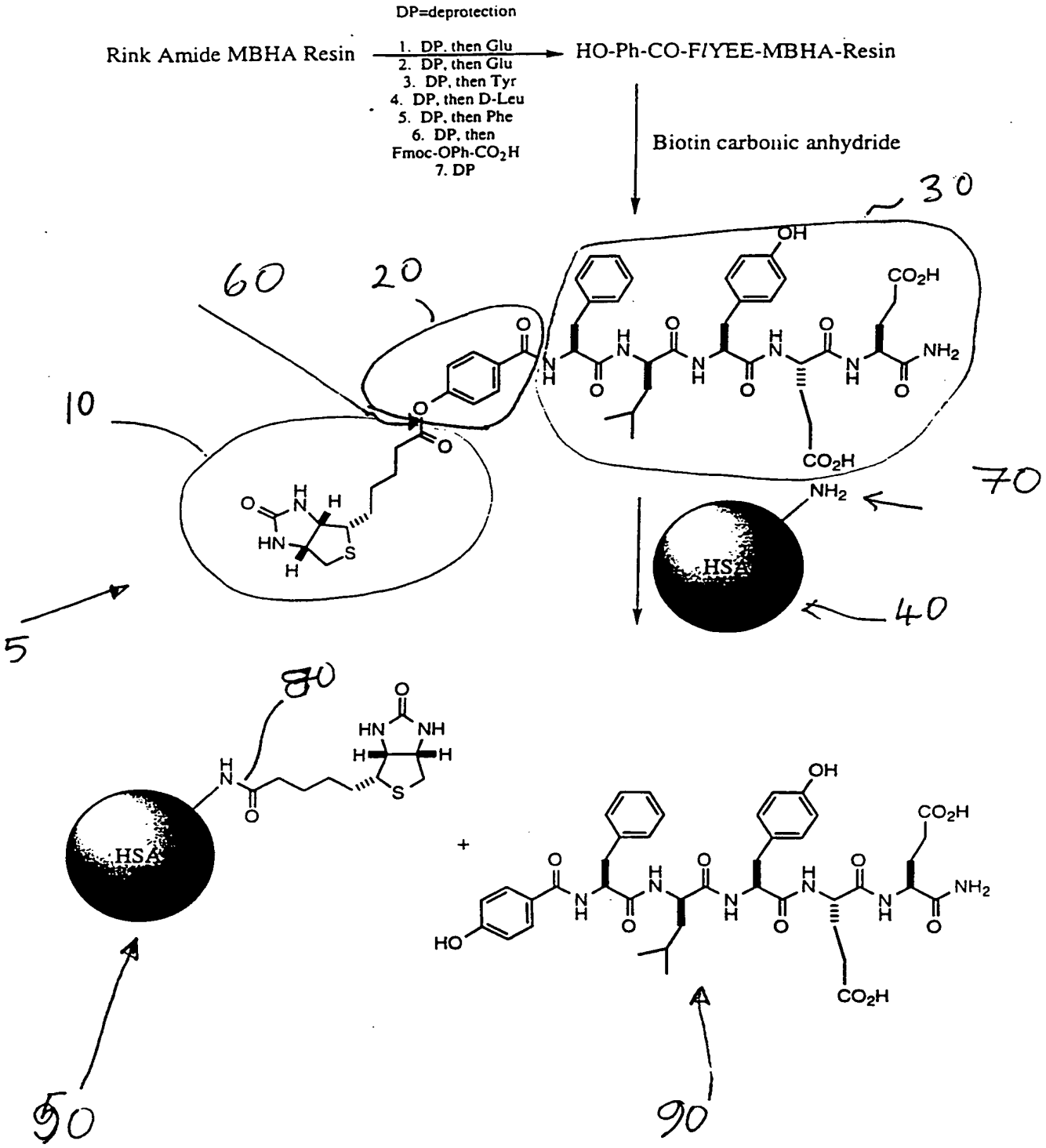

DP=deprotection
Rink Amide MBHA Resin
1. DP, then Glu
2. DP, then Glu
3. DP, then Tyr
4. DP, then D-Leu
5. DP, then Phe
6. DP, then Fmoc-OPh-CO2H
7. DP
HO-Ph-CO-FIYEE-MBHA-Resin
Biotin carbonic anhydride
30
60
20
10
70
40
5
80
50
90
HSA
OH
CO2H
NH2
HN
NH
S
HO

FIG. 2

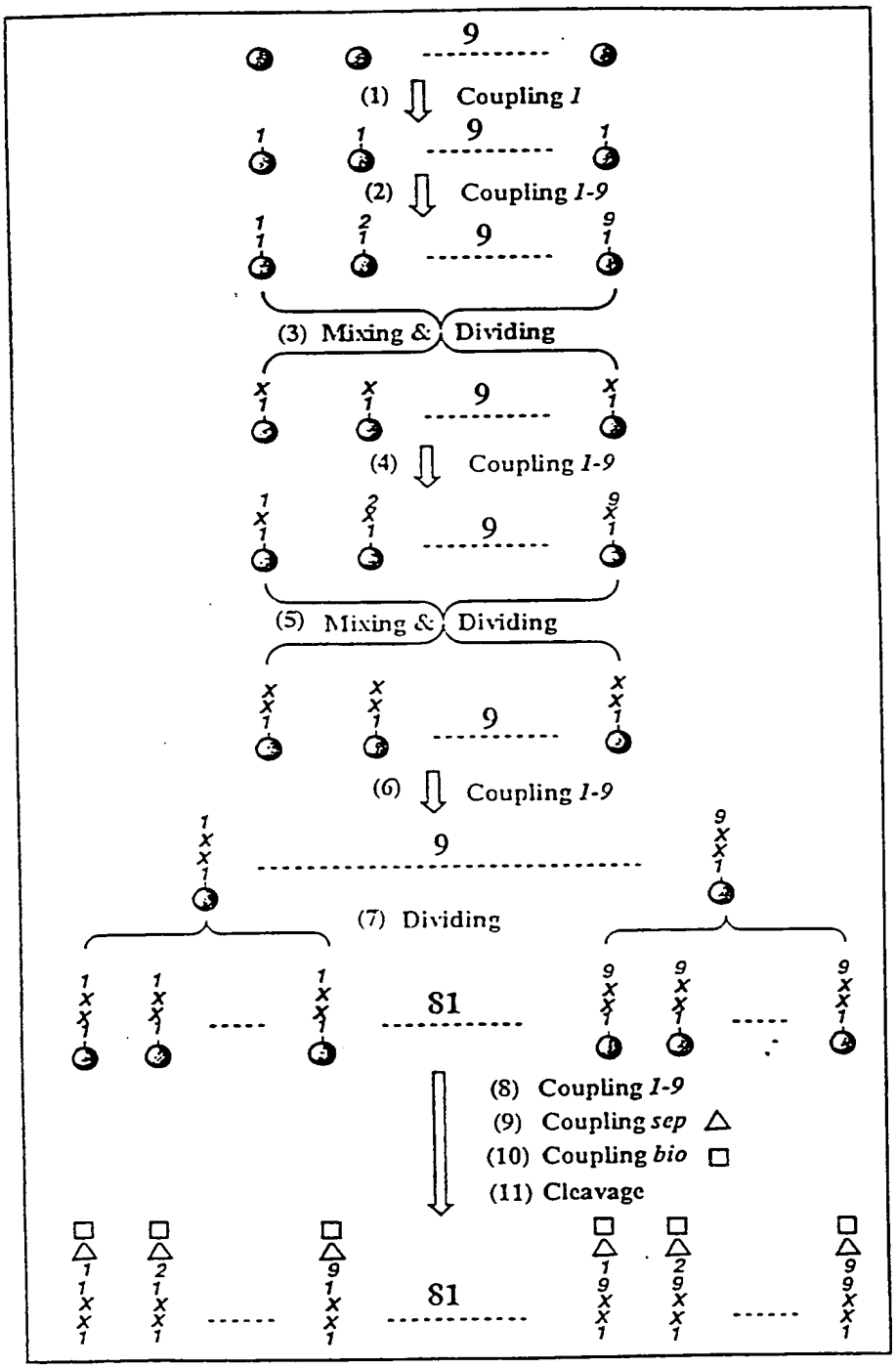

9
(1) Coupling 1
9
(2) Coupling 1-9
9
(3) Mixing & Dividing
9
(4) Coupling 1-9
9
(5) Mixing & Dividing
9
(6) Coupling 1-9
9
(7) Dividing
81
(8) Coupling 1-9
(9) Coupling sep
(10) Coupling bio
(11) Cleavage
81

FIG. 3

FIG.4

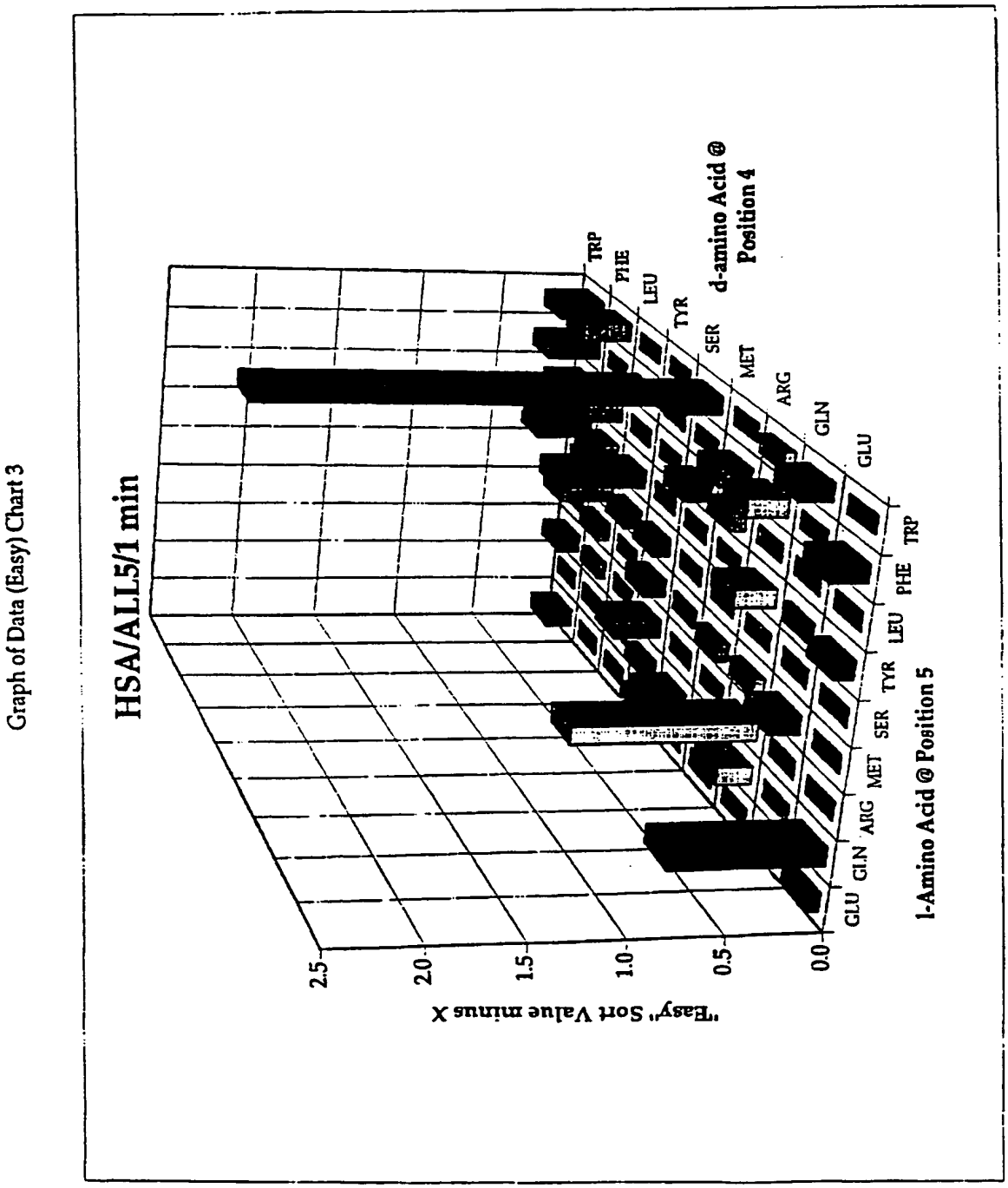
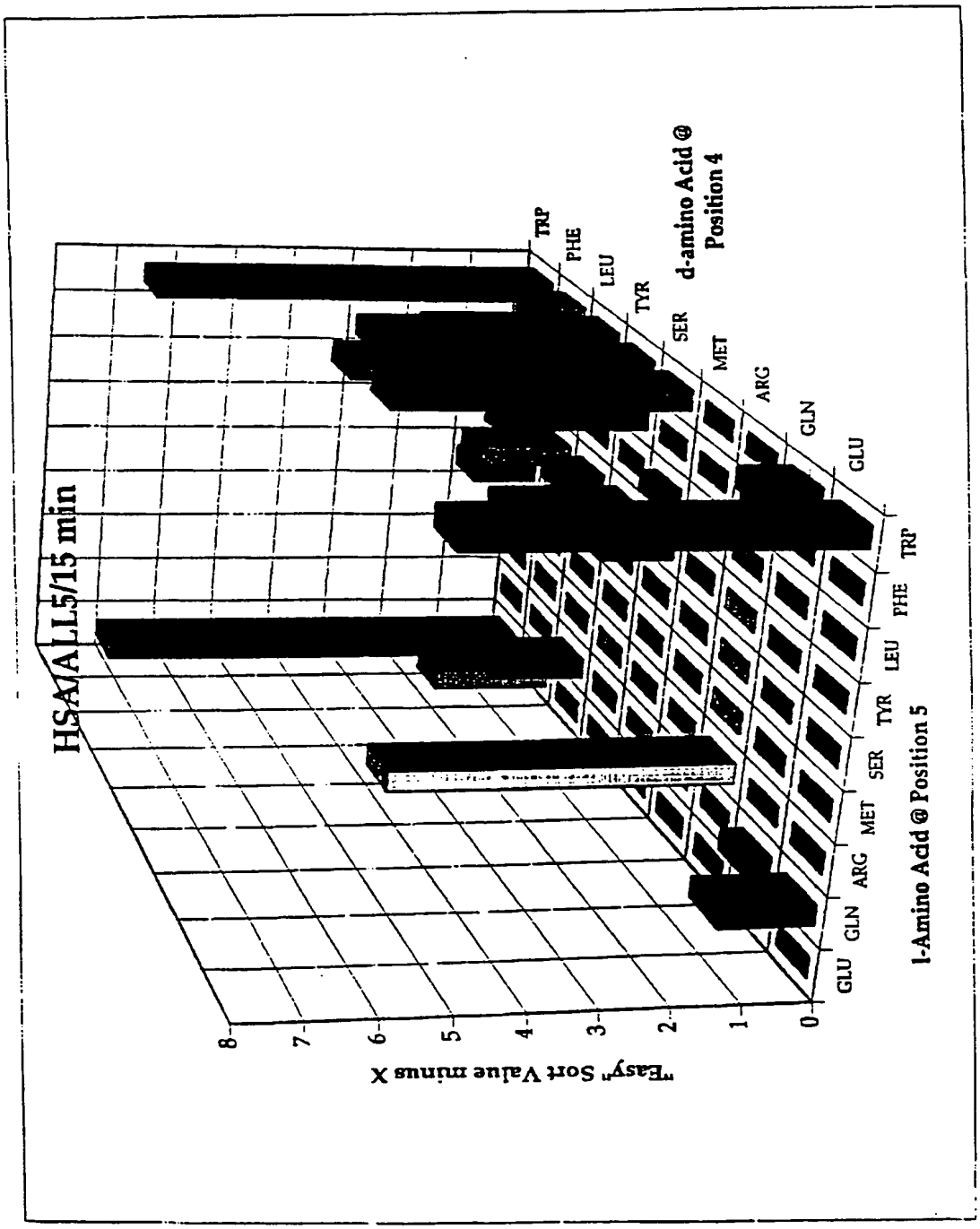

Graph of Data (Easy) Chart 3
HSA/ALL5/1 min
"Easy" Sort Value minus X
2.5
2.0
1.5
1.0
0.5
0.0
GLU
GLN
ARG
MET
SER
TYR
LEU
PHE
TRP
l-Amino Acid @ Position 5
TRP
PHE
LEU
TYR
SER
MET
ARG
GLN
GLU
d-amino Acid @ Position 4

FIG.5


Graph of Data (Easy) Chart 3
HSAALL5/15 min
"Easy" Sort Value minus X
0
1
2
3
4
5
6
7
8
GLU
GLN
ARG
MET
SER
TYR
LEU
PHE
TRP
1-Amino Acid @ Position 5
TRP
PHE
LEU
TYR
SER
MET
ARG
GLN
GLU
d-amino Acid @ Position 4

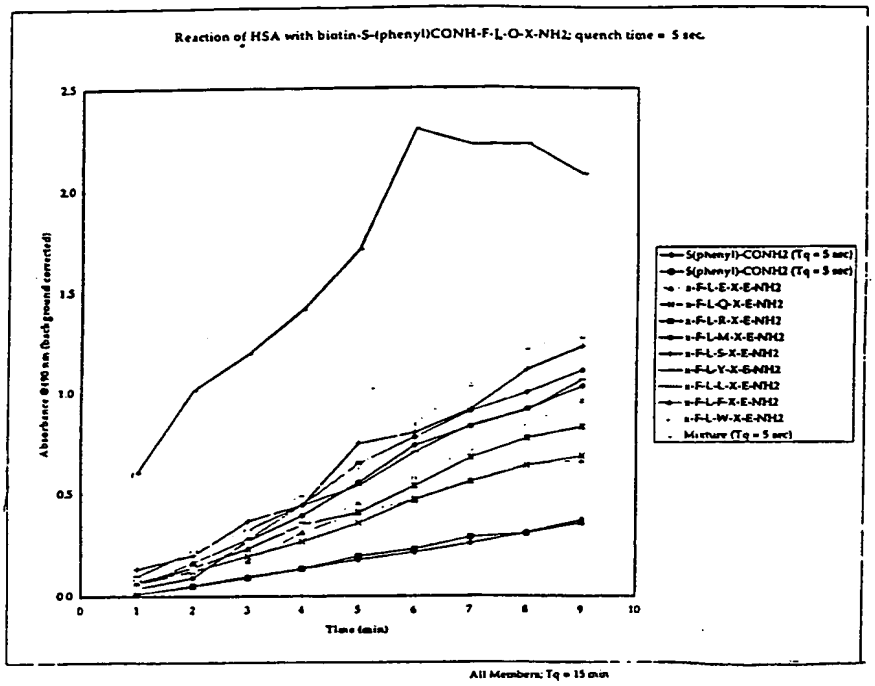

Reaction of HSA with biotin-S-(phenyl)CONH-F-L-O-X-NH2; quench time = 5 sec.
Absorbance @490 nm (background corrected)
Time (min)
S(phenyl)-CONH2 (Tq = 5 sec)
S(phenyl)-CONH2 (Tq = 5 sec)
s-F-L-E-X-E-NH2
s-F-L-Q-X-E-NH2
s-F-L-R-X-E-NH2
s-F-L-M-X-E-NH2
s-F-L-S-X-E-NH2
s-F-L-Y-X-E-NH2
s-F-L-L-X-E-NH2
s-F-L-F-X-E-NH2
s-F-L-W-X-E-NH2
Mixture (Tq = 5 sec)
All Members; Tq = 15 min

FIG. 6a

FIG.6b

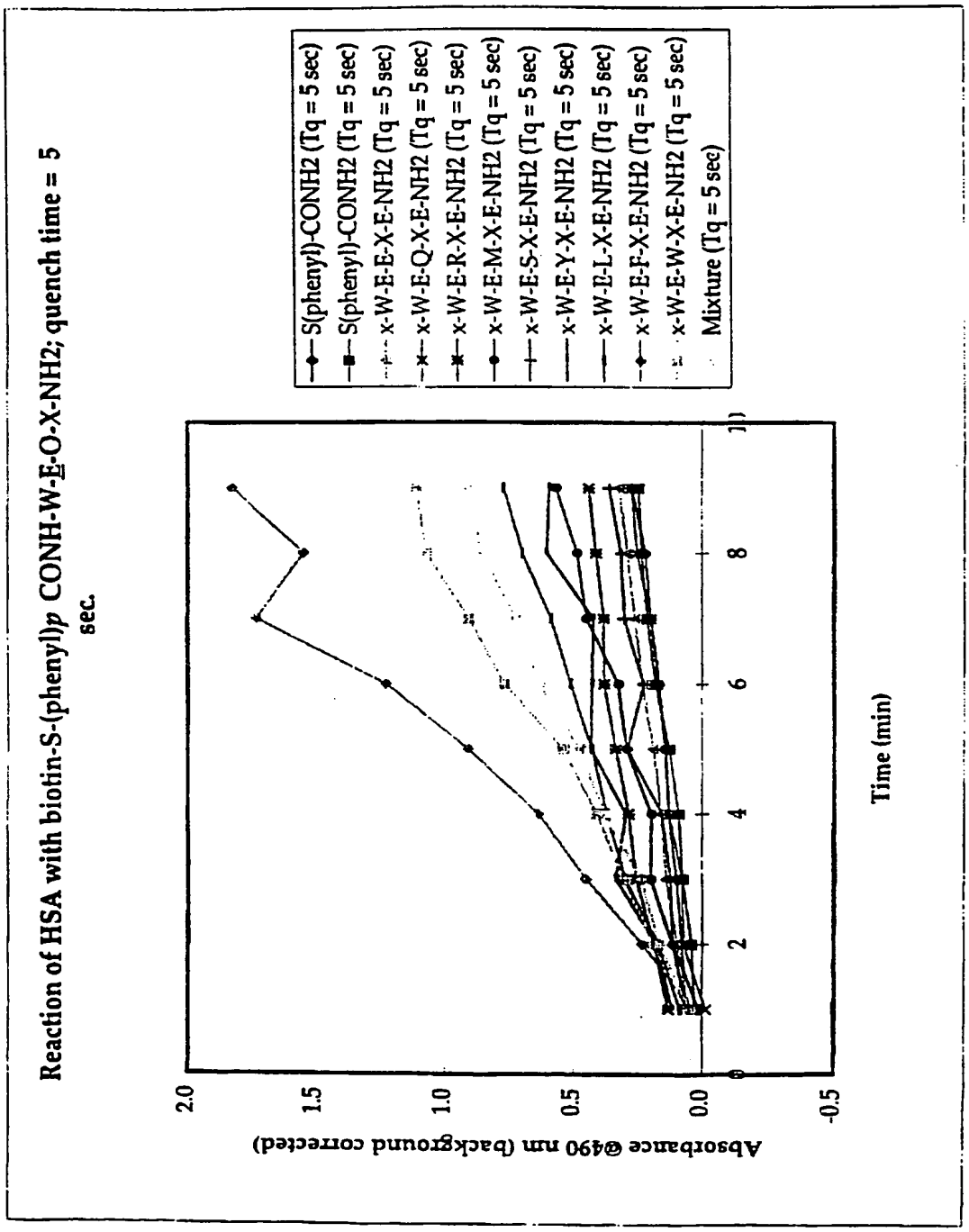
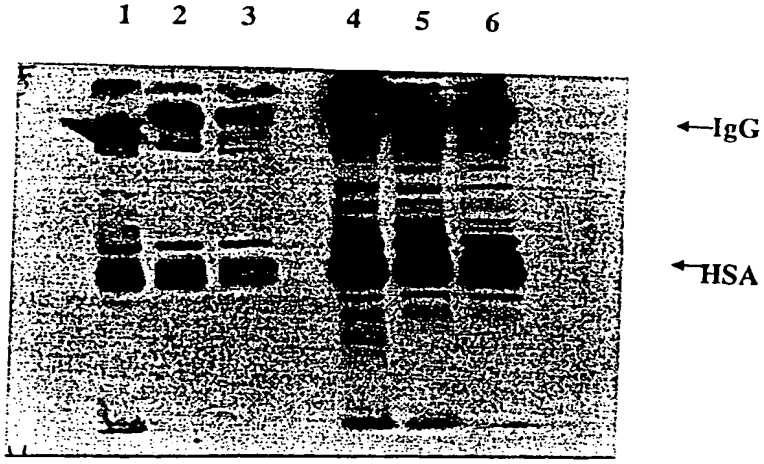

Reaction of HSA with biotin-S-(phenyl)p CONH-W-E-O-X-NH2; quench time = 5 sec.
All Members; Tq = 5 sec Chart 1
S(phenyl)-CONH2 (Tq = 5 sec)
S(phenyl)-CONH2 (Tq = 5 sec)
x-W-E-E-X-E-NH2 (Tq = 5 sec)
x-W-E-Q-X-E-NH2 (Tq = 5 sec)
x-W-E-R-X-E-NH2 (Tq = 5 sec)
x-W-E-M-X-E-NH2 (Tq = 5 sec)
x-W-E-S-X-E-NH2 (Tq = 5 sec)
x-W-E-Y-X-E-NH2 (Tq = 5 sec)
x-W-E-L-X-E-NH2 (Tq = 5 sec)
x-W-E-F-X-E-NH2 (Tq = 5 sec)
x-W-E-W-X-E-NH2 (Tq = 5 sec)
Mixture (Tq = 5 sec)
Absorbance @490 nm (background corrected)
2.0
1.5
1.0
0.5
0.0
-0.5
0
2
4
6
8
Time (min)

FIG. 7


1 2 3 4 5 6
IgG
HSA

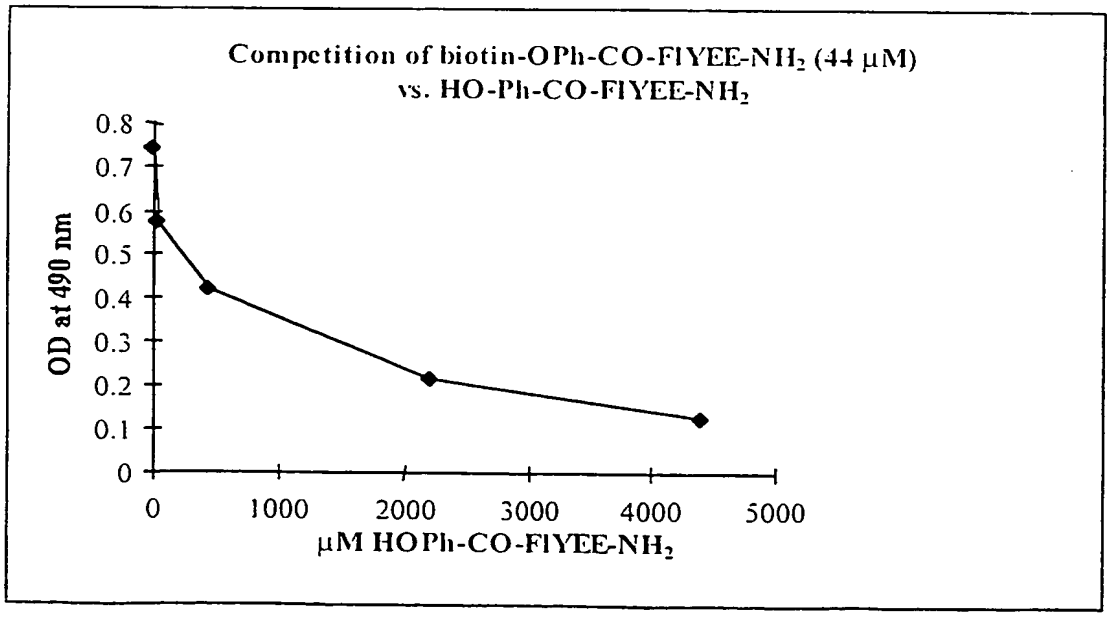
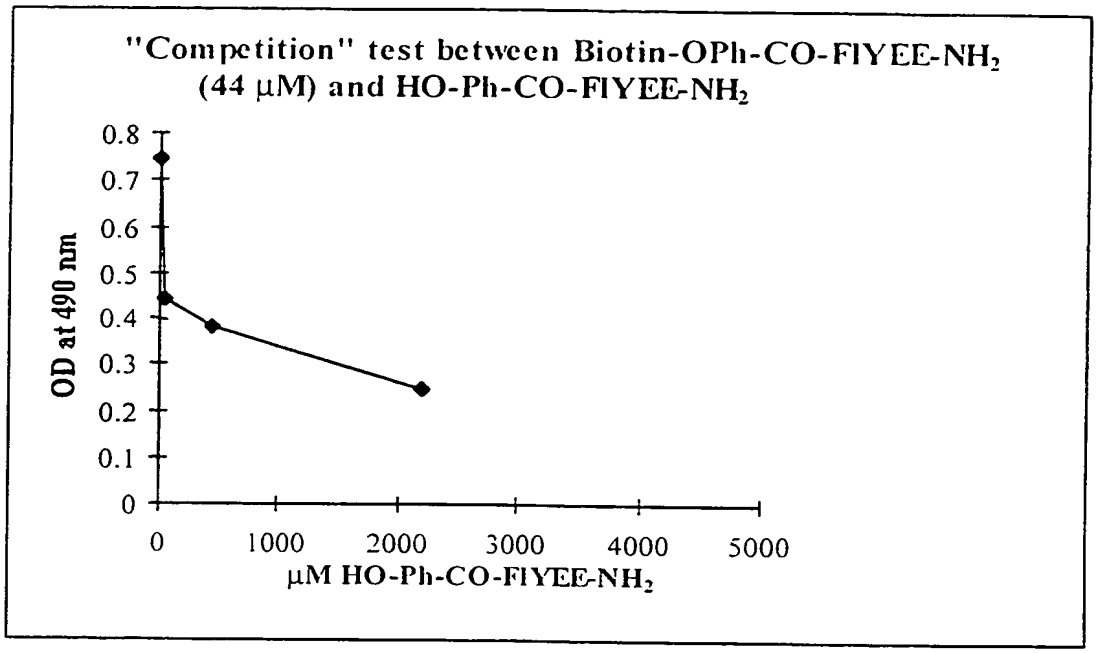

Competition of biotin-OPh-CO-FIYEE-NH2 (44 µM)
vs. HO-Ph-CO-FIYEE-NH2
OD at 490 nm
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1000
2000
3000
4000
5000
µM HOPh-CO-FIYEE-NH2

FIG. 8

FIG. 9


"Competition" test between Biotin-OPh-CO-FIYEE-NH2
(44 µM) and HO-Ph-CO-FIYEE-NH2
OD at 490 nm
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1000
2000
3000
4000
5000
µM HO-Ph-CO-FIYEE-NH2

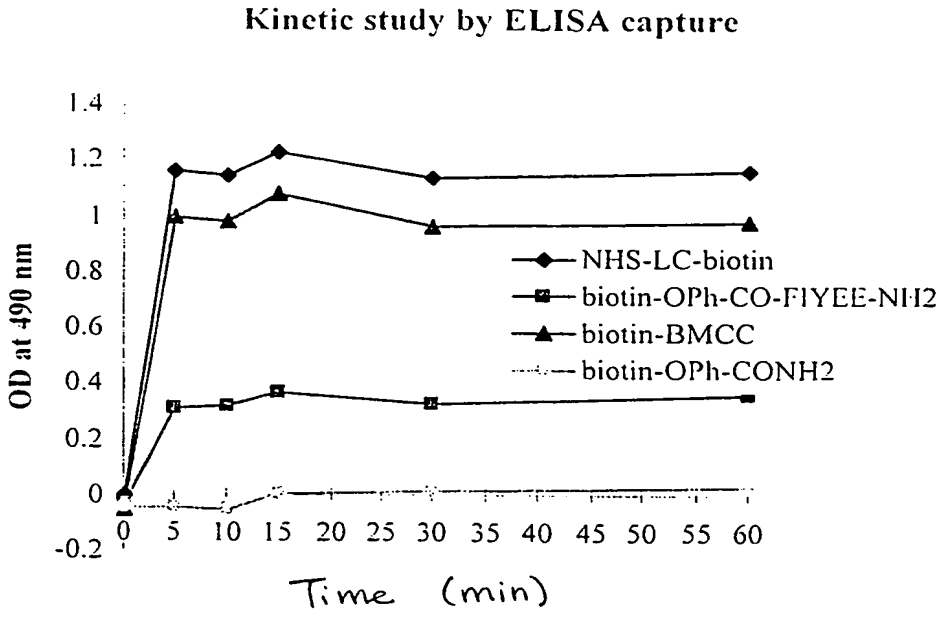

Kinetic study by ELISA capture
OD at 490 nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (min)
NHS-LC-biotin
biotin-OPh-CO-FIYEE-NH2
biotin-BMCC
biotin-OPh-CONH2

FIG. 10

FIG. 11

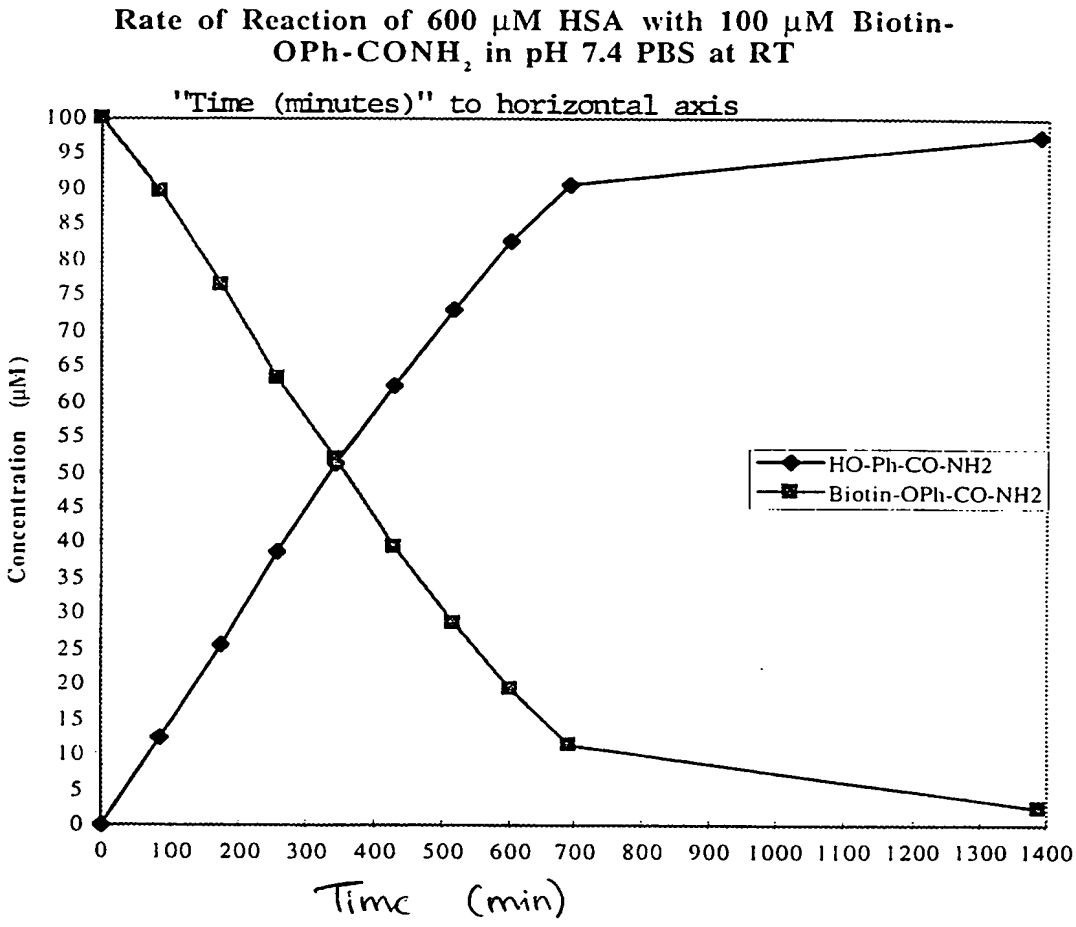

Rate of Reaction of 600 μM HSA with 100 μM Biotin-OPh-CONH2 in pH 7.4 PBS at RT
"Time (minutes)" to horizontal axis
Concentration (μM)
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
Time (min)
HO-Ph-CO-NH2
Biotin-OPh-CO-NH2

FIG. 12

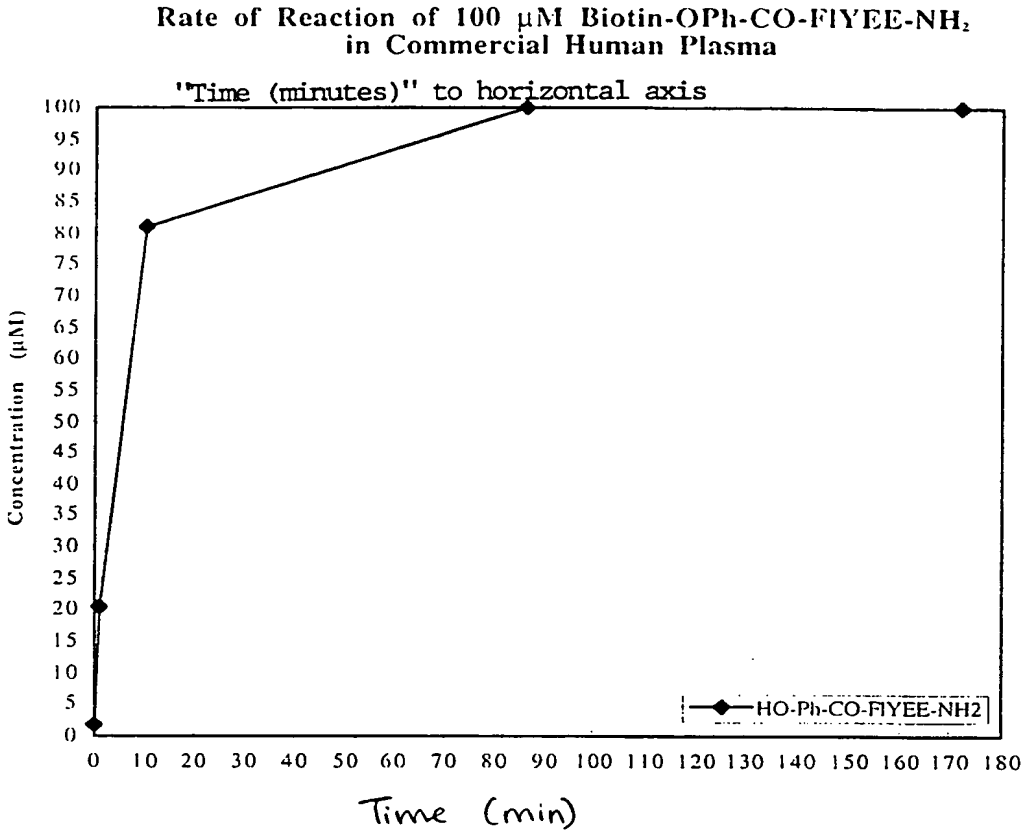

Rate of Reaction of 100 μM Biotin-OPh-CO-FIYEE-NH2
in Commercial Human Plasma
"Time (minutes)" to horizontal axis
Concentration (μM)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
Time (min)
HO-Ph-CO-FIYEE-NH2

FIG. 13

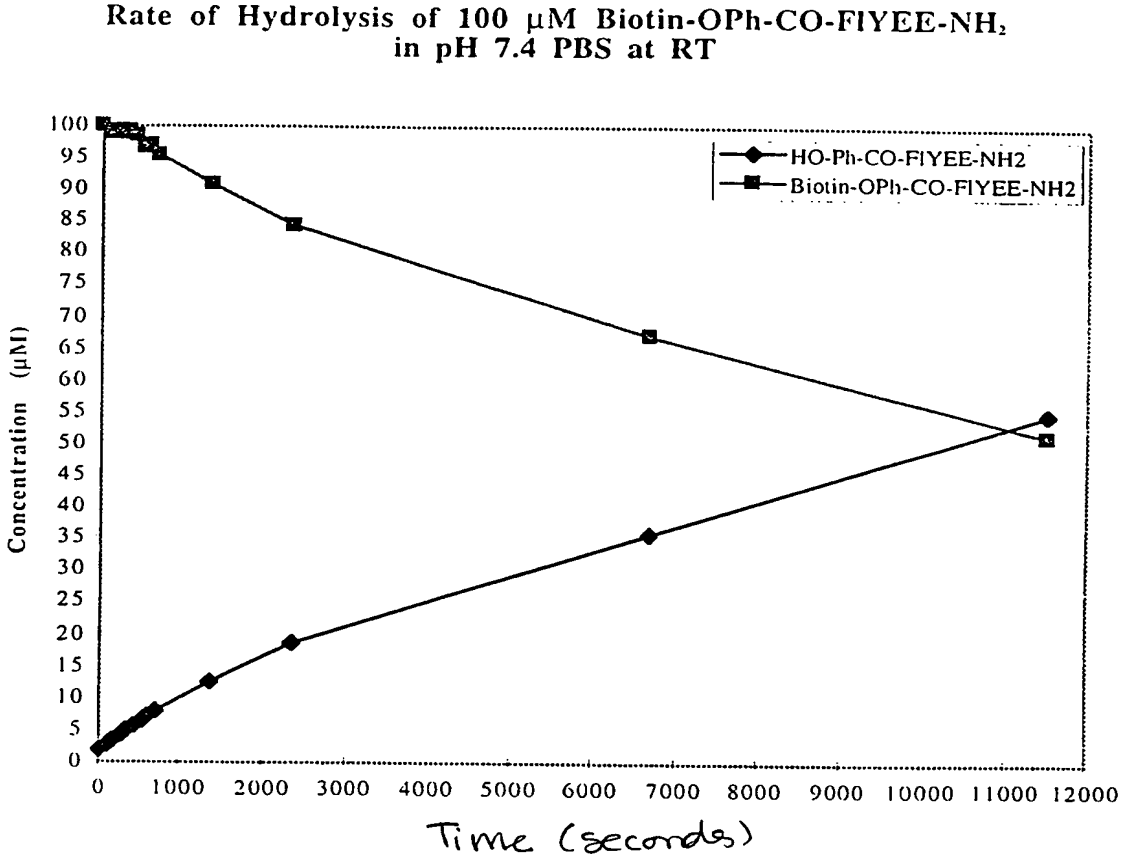
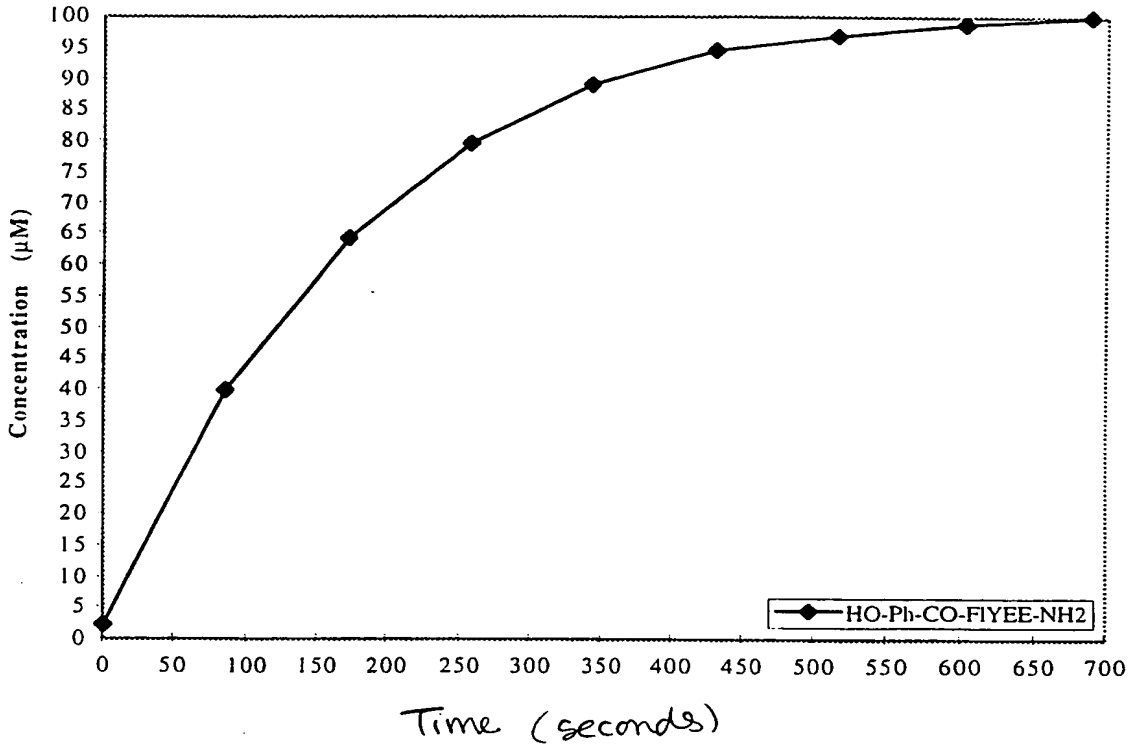

Rate of Hydrolysis of 100 µM Biotin-OPh-CO-FIYEE-NH2
in pH 7.4 PBS at RT
HO-Ph-CO-FIYEE-NH2
Biotin-OPh-CO-FIYEE-NH2
Concentration (µM)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
12000
Time (seconds)

FIG. 14

Rate of Reaction of 100 μM Biotin-OPh-CO-FlYEE-NH_2 with 600 μM HSA at RT



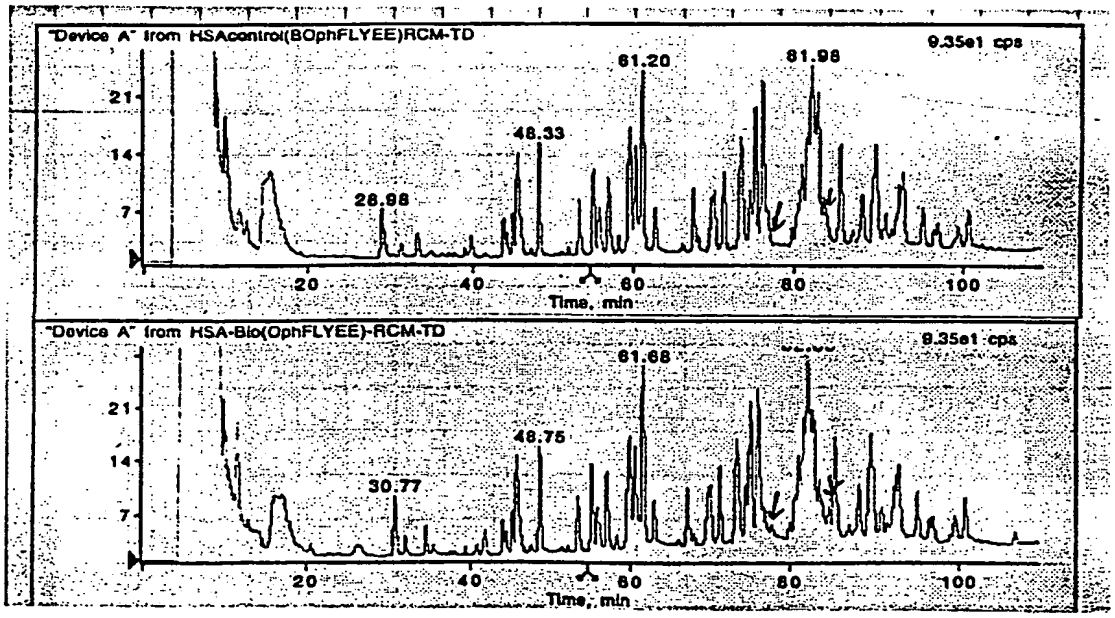
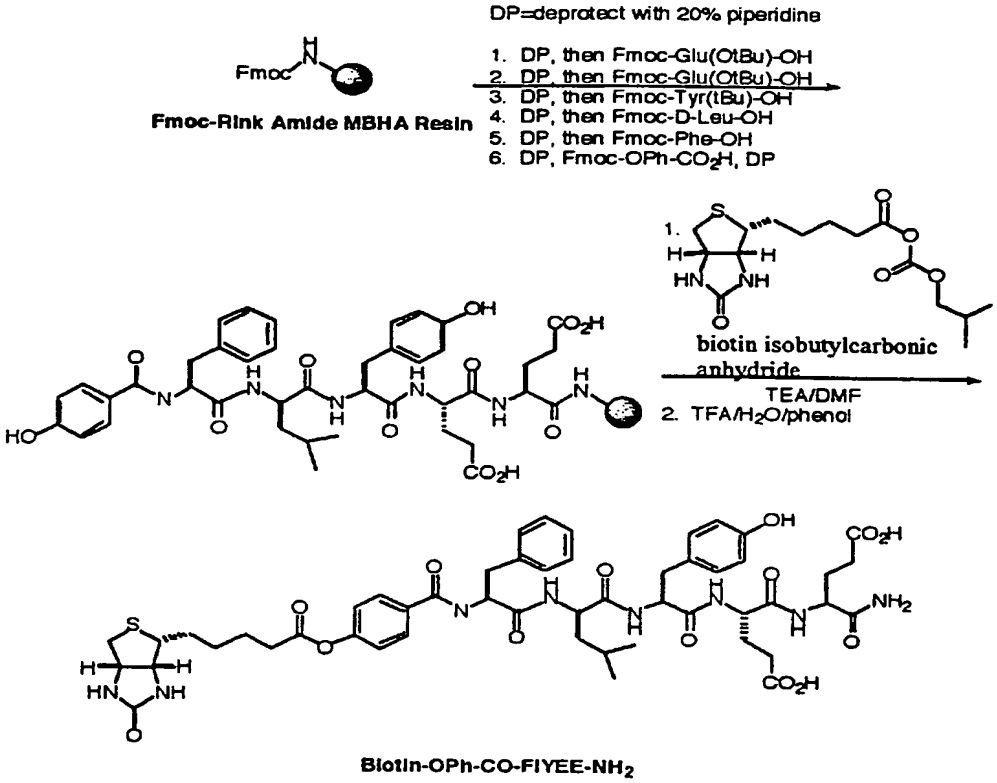


FIG. 15



Biotin-OPh-CO-FIYEE-NH_2

FIG. 16

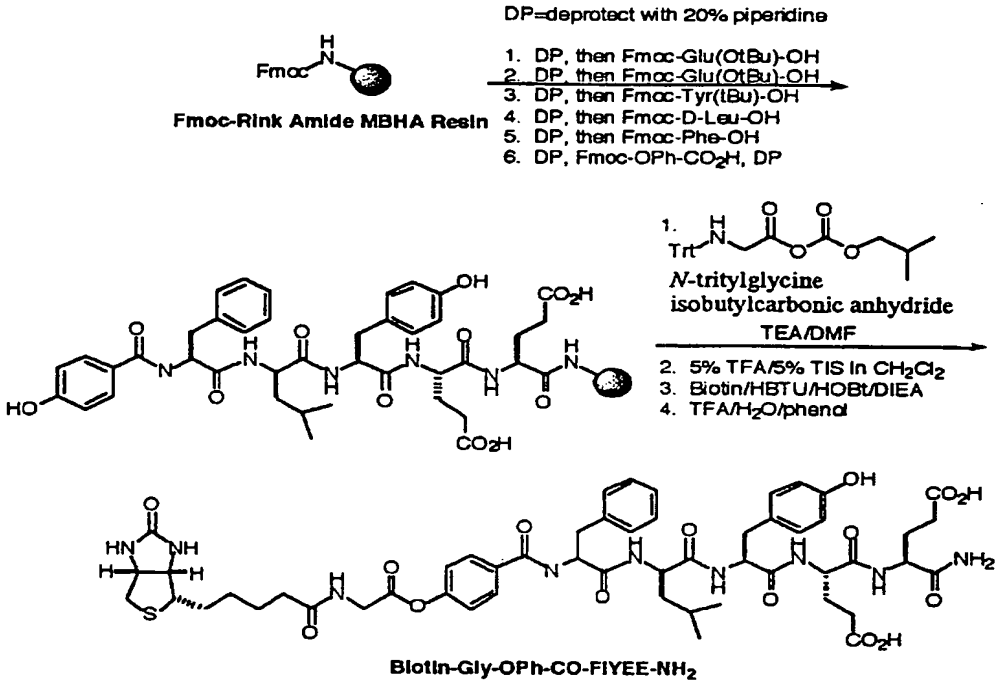

DP=deprotect with 20% piperidine
1. DP, then Fmoc-Glu(OtBu)-OH
2. DP, then Fmoc-Glu(OtBu)-OH
3. DP, then Fmoc-Tyr(tBu)-OH
4. DP, then Fmoc-D-Leu-OH
5. DP, then Fmoc-Phe-OH
6. DP, Fmoc-OPh-CO2H, DP
Fmoc-Rink Amide MBHA Resin
1.
N-tritylglycine
isobutylcarbonic anhydride
TEA/DMF
2. 5% TFA/5% TIS in CH2Cl2
3. Biotin/HBTU/HOBt/DIEA
4. TFA/H2O/phenol
Biotin-Gly-OPh-CO-FIYEE-NH2

FIG. 17

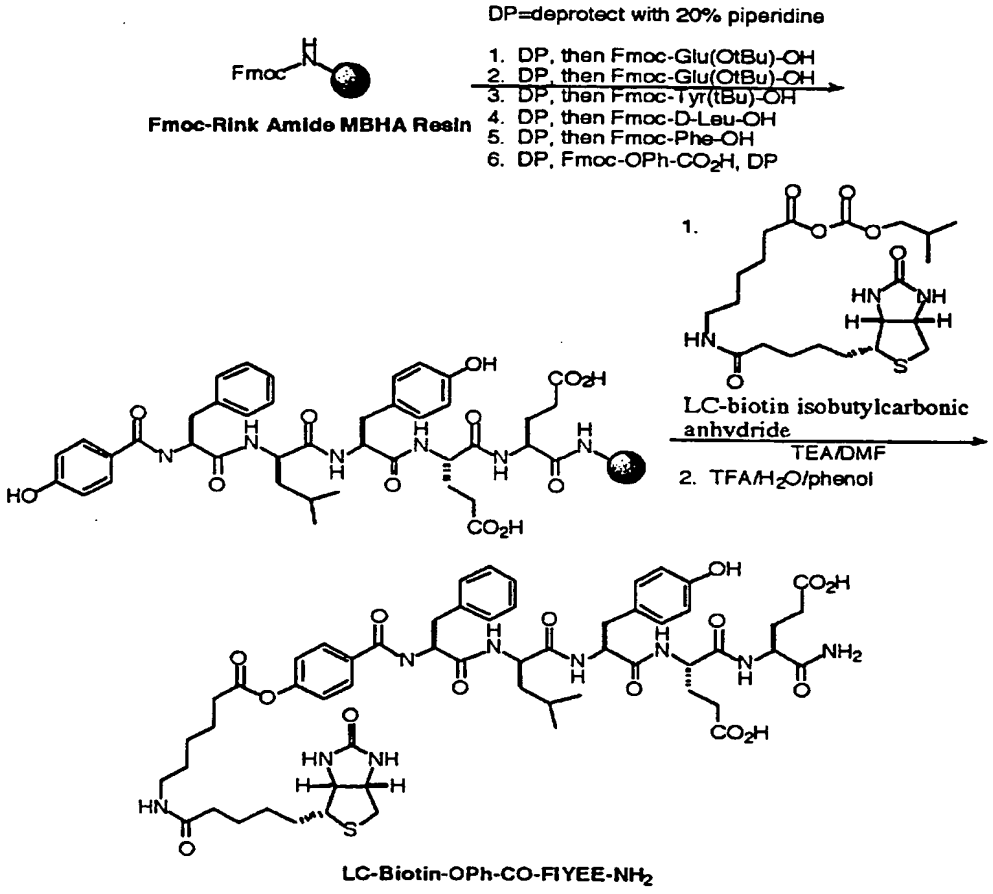

DP=deprotect with 20% piperidine
Fmoc
Fmoc-Rink Amide MBHA Resin
1. DP, then Fmoc-Glu(OtBu)-OH
2. DP, then Fmoc-Glu(OtBu)-OH
3. DP, then Fmoc-Tyr(tBu)-OH
4. DP, then Fmoc-D-Leu-OH
5. DP, then Fmoc-Phe-OH
6. DP, Fmoc-OPh-CO2H, DP
1.
LC-biotin isobutylcarbonic anhydride
TEA/DMF
2. TFA/H2O/phenol
LC-Biotin-OPh-CO-FIYEE-NH2

FIG. 18

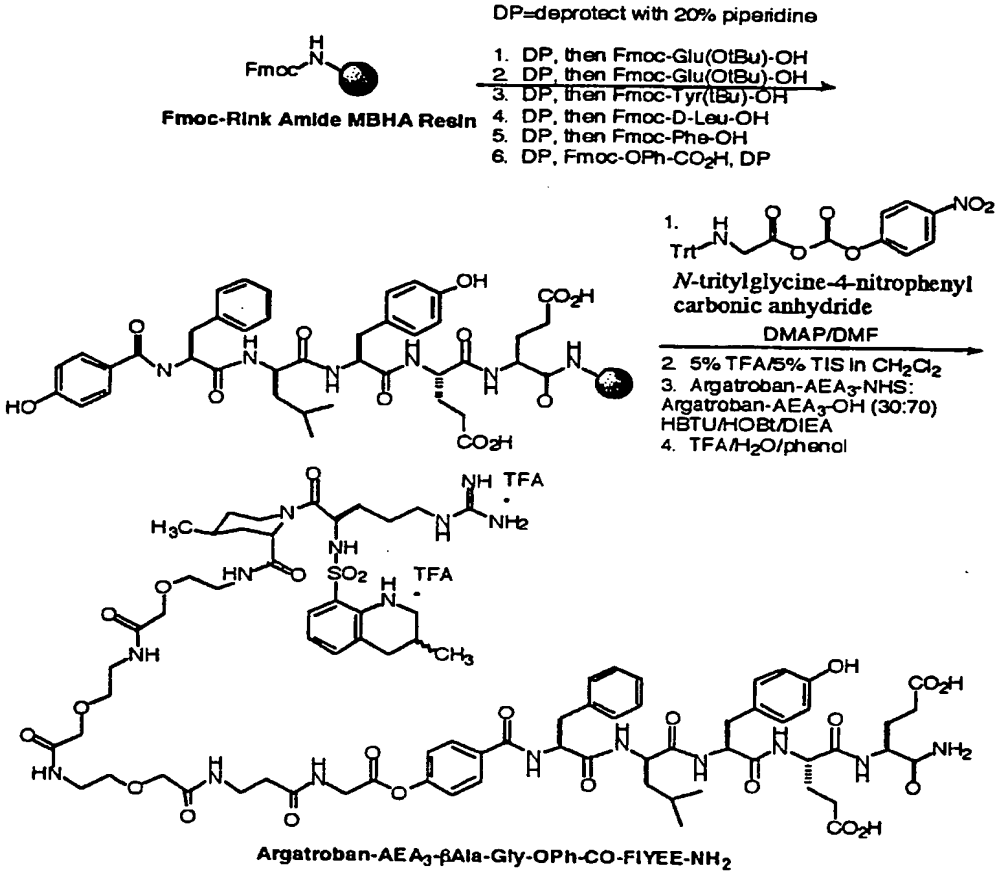

DP=deprotect with 20% piperidine
Fmoc-Rink Amide MBHA Resin
1. DP, then Fmoc-Glu(OtBu)-OH
2. DP, then Fmoc-Glu(OtBu)-OH
3. DP, then Fmoc-Tyr(tBu)-OH
4. DP, then Fmoc-D-Leu-OH
5. DP, then Fmoc-Phe-OH
6. DP, Fmoc-OPh-CO2H, DP
1.
N-tritylglycine-4-nitrophenyl carbonic anhydride
DMAP/DMF
2. 5% TFA/5% TIS in CH2Cl2
3. Argatroban-AEA3-NHS: Argatroban-AEA3-OH (30:70) HBTU/HOBt/DIEA
4. TFA/H2O/phenol
TFA
TFA
Argatroban-AEA3-βAla-Gly-OPh-CO-FIYEE-NH2

FIG. 19

Synthetic Schemes

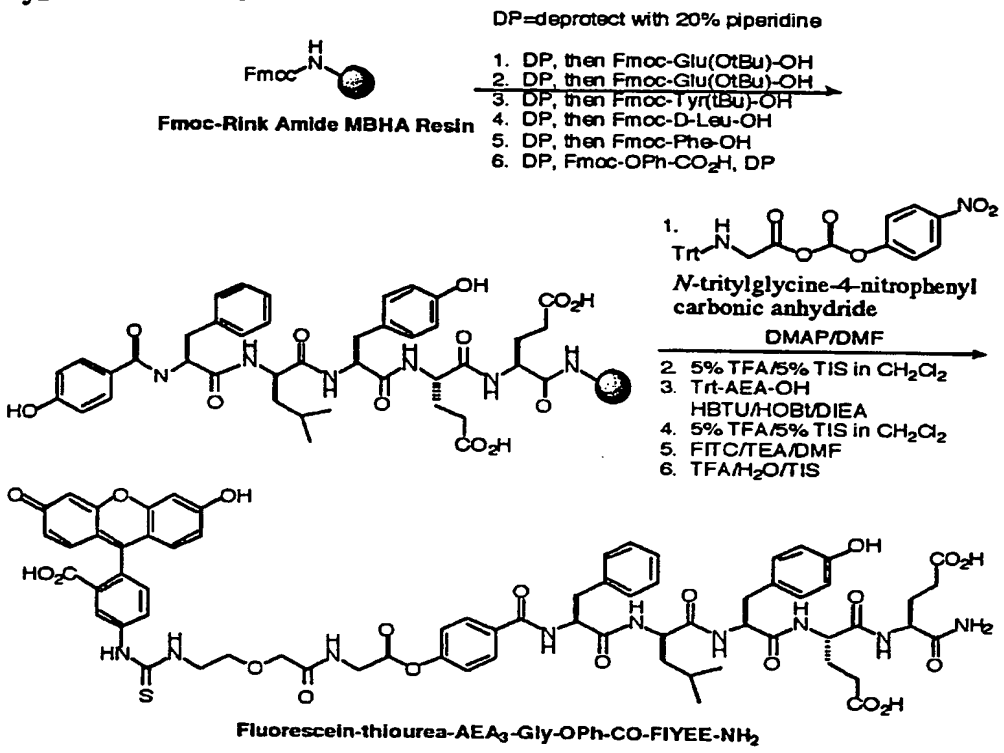


FIG. 20